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RELATIONSHIP OF BLOOD PLASMA VITAMIN C LEVEL TO GINGIVAL AND PERIODONTAL DISEASE[1]

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In the years since vitamins were first discovered much has been learned of many of the vitamins, but the relationships between them and oral health have remained obscure. It is a pleasant and worthwhile undertaking, therefore, to try to add even a little to the store of knowledge of those relationships.

Vitamin C is one of several vitamins which may be necessary for maintenance of oral health. The oral effects of a deficiency of vitamin C may be confused with, and complicated by, a number of other factors. Improper mouth hygiene, deposits on the teeth, faulty tooth brushing, malocclusion, and other local causes may be partly or wholly responsible in any one case for the symptoms which may seem to be chargeable to lack of vitamin C. Deficiencies of several vitamins at once might produce changes in the mouth comparable to changes caused by more severe deficiencies of vitamin C alone.

For these reasons, the study of the relationship between vitamin C and the condition of the gingival and periodontal tissues should depend on data from a large number of cases. If the number is large enough it may be possible to discount many variables and learn the effects of low levels of vitamin C alone. In the present paper records of 1396 patients are considered in an attempt to determine what correlation there may be between vitamin C levels in the blood plasma and the common afflictions, gingivitis and periodontal disease.

REVIEW

When guinea pigs are kept on a diet deficient in vitamin C, changes occur in the gingiva, periodontal membrane and alveolar bone similar to the changes observed in mouths of humans suffering from gingivitis and periodontal disease. The same changes occur less quickly and less severely in guinea pigs when the diet is low, but not lacking in vitamin C. This has been shown by Howe, Westin, Wohlbach, Hojer, Day, Fish, Harris, Boyle and Bessey (1 to 11), each substantially confirming the results reported by the others.

It is not clear that the same thing is true of human subjects. Crandon, Lund and Dill (12) reported the results of an experiment on a human subject whose mouth was in good condition after 6 months on a diet entirely lacking in vitamin C. One slight gingival hemorrhage occurred after a fatigue test, but otherwise there was no sign of gingivitis and the only periodontal change noted was

[1] Read at the 20th General Meeting of the International Association for Dental Research, New York, March 14 and 15, 1942 (*J. D. Res.*, **21**: 330, 1942). Received for publication March 26, 1942.

in x-ray pictures showing slight interruptions in the lamina dura at the end of the experiment.

Tishler (13), Freeman and Glass (14), Kirkpatrick (15), and Radusch (16) found no significant relationship between vitamin C and mouth conditions. On the other hand, Boyle, Bessey and Wohlbach (17) reported definite correlations between low vitamin C level and rarefactions of the alveolar bone not attributable to local causes. Pelzer (18) found a tendency in vitamin C deficient cases toward bleeding, cyanosis and bloating of the gingivae. Westin (19) reported marginal atrophy of the alveolar bone in human scurvy cases. Weisberger, Young and Morse (20) reported that all of their cases whose blood vitamin C levels were low were afflicted with marginal gingivitis. They noted improvement upon administration of 2000 mg. of the vitamin over a period of 2 weeks.

Sorrin (21) and Fitzsimmons (22) found that systemic or dietary factors were operative in only a few of the cases in the groups studied, and that local causes were operative in most.

Hanke (23, 24, 25) and Hawkins (26) have reported improvement in gingival conditions upon the addition of ample orange juice to the diet. These reports are not necessarily pertinent to the question of whether lack of vitamin C is an etiologic factor in gingivitis and periodontal disease, because it is possible that administration of excess vitamins might produce improvement in the mouth tissues where the disturbance was due to other things than vitamin deficiency. Also it is possible that the orange juice contains some active ingredient other than the vitamin C.

The literature indicates that vitamin C deficiency may be entirely responsible for gingival troubles in some cases, and partly responsible in many, but that many cases of gingivitis will clear up entirely if the local causes are removed. There may be a lowered tissue resistance traceable to vitamin deficiency. The diversity of the published opinions may be due to differences in methods, to different standards by which mouth conditions are judged, or possibly to the use of relatively small numbers of patients.

METHODS

Over a period of 24 months, records have been kept showing the gingival condition, the periodontal condition, and the blood plasma vitamin C levels of 1396 patients coming into the clinic of the Dental School. The patients were not selected on the basis of gingival condition or plasma vitamin C level or in any other way, except that the vitamin C determination was made on some because the gingival condition raised a question of possible deficiency. Unfortunately, there was no uniformity in the vitamin C levels in these particular cases. The inclusion of such cases raises somewhat the proportion of those having severe gingivitis.

Each case was examined by one or another of 4 instructors in the diagnosis clinic. The gingival and periodontal conditions were noted. The vitamin C determination was then made by a technician who was not informed of the results of the examination. The results of the vitamin determination were not known until later, when they were recorded on the examination chart by the departmental secretary.

The degree of gingival inflammation was designated by the figures 1, 2 or 3 on the record; 1 indicating no visible inflammation, 2 a moderate amount, and 3 severe inflammation. This system of grading depends on the judgment of the observer, so that there probably is some overlap between the cases marked 2 and those marked 3. There is less chance that

cases marked 1, where there was no visible inflammation, should be confused with cases marked 2 or 3 where inflammation was clearly present.

The periodontal condition was recorded in a like manner. The figure 1 indicated that there were no abnormally deep gingival crevices, 2 that the pockets were very few or shallow, or both, and 3 that there were many deep pockets. Three mm. was considered a moderate pocket depth. Pockets 4 or more mm. deep called for a designation of 3 if there were more than 1 or 2 such pockets. The recording of the periodontal condition was checked by reference to roentgenograms where possible, but no attempt is made in this study to distinguish between the diffuse, and the deeper and more local forms of periodontosis. As with the recordings of gingivitis, the periodontosis records depend on personal observations, so that there is probability of confusion between the 2 and 3 cases, but less probability of confusion between these and the cases marked 1.

The age of the patient and the date of the examination were noted and record was made of the presence of calculus, malocclusion or any other condition which might provide a local cause of gingival disturbance. It is possible that this notation was not made in all cases where it should have been. If there was any specific gingival disturbance present, such as Vincent's infection, the fact was noted. Such cases were thought to be not comparable and are not included with the others in this study.

Record was also made of what the patient last had to eat and of the time elapsed since he ate it. This information was of value in excluding cases whose vitamin C level may have been raised because of recent intake of vitamin rich foods. The records of patients who had partaken of citrus fruits or tomatoes within 3½ hours before the vitamin determination was made were later grouped separately and are not included in the other groups of cases.

The blood plasma vitamin C level was determined by the micro-method of Farmer and Abt (27). In this determination, 0.2 cc. of blood is drawn from the finger into a tube in which has been placed a small amount of lithium oxalate. The blood is centrifuged to free the plasma of cells. The plasma is then drawn off with a pipette and placed in another tube, where m-phosphoric acid is added to precipitate the proteins. It is again centrifuged and the protein-free fluid drawn off and titrated with 2-6-dichlorphenolindophenol. The results are expressed as mg. of ascorbic acid per 100 cc. of blood plasma.

In many cases several vitamin C determinations were made on different dates, but only the determinations made on the same day as the recorded examinations are considered here, and no repeat for any patient is included in the figures.

In preparing the data for study, the pertinent figures were transcribed from the examination charts to small cards which could then be rearranged according to any particular phase of the question.

RESULTS

It was found that the blood plasma vitamin C determinations ranged from 0.04 mg. per 100 cc. of plasma to 2.16 mg. per 100 cc. This range was substantially the same for all types of cases. The lowest reading was found in a patient under 20 with no gingivitis, and the highest was in a middle aged patient with moderate gingivitis. By inspection no correlation could be discovered between the vitamin C value and the condition of the mouth. Only when the cases were grouped in large numbers did any differences appear. These differences were small. In every case the differences between the means, medians, or modes of different groups were small compared to the ranges of individual readings within each group.

1. *Vitamin C and Gingivitis*

After removing the records of those patients who had taken citrus fruits or other sources of vitamin C within 3½ hours, the remaining cases were sorted

into 3 groups according to the gingivitis record of each. The cases within each group were then arranged in the increasing order of the vitamin C values. In Table I the total numbers of cases, the range of vitamin C readings and the mean, median and mode for each group are stated.

The groups, 0.20-0.40 and 0.40–0.60, were used to express the mode because it was found that steps of that magnitude were necessary to avoid minor and confusing variations in the curves obtained.

Because of the undue importance which the mean determination gives to scattered very high vitamin C levels, it is thought that the median is the most reliable index of the vitamin status of each group. If the median of the gingivitis 1 group is taken as normal, there are in this group as many cases above 0.52 mg. as there are below this level. A division of the other groups into cases above and below 0.52 mg. gives the results shown in Table II.

TABLE I

	NO. CASES	RANGE	VIT. C-LEVEL IN MG. PER 100 CC. PLASMA		
			Mean	Median	Mode
Gingivitis 1	743	0.04–1.92	0.56	0.52	0.40–0.60
Gingivitis 2	410	0.08–2.16	0.50	0.40	0.20–0.40
Gingivitis 3	107	0.08–1.96	0.49	0.40	0.20–0.40

TABLE II

	PER CENT CASES ABOVE THE NORM (0.52 MG. VIT. C)	PER CENT CASES BELOW THE NORM (0.52 MG. VIT. C)
No gingivitis (1)	50	50
Moderate gingivitis (2)	36.3	63.7
Severe gingivitis (3)	34.9	65.1

These figures show that there is some connection between the vitamin C level and the condition of the gingivae, but in view of the wide and almost identical ranges of the groups, that is, from 0.04 or 0.08 to 1.98 or 2.16, it is clear that the vitamin C level is but one factor among others. There is clearly a tendency either for low vitamin C cases to have gingivitis, or for gingivitis cases to have a low vitamin C, but whether the low vitamin C is responsible for certain cases and other factors for other cases, or whether all factors tend to operate in most of the cases cannot be ascertained from these figures. The trend of the figures may indicate only a correlation between neglect of the mouth and neglect of the diet.

An attempt to eliminate at least some of the other factors was made by taking out of the groups those cases in which there was note of calculus deposits, malocclusion, and the like which, in themselves, may constitute causes of gingivitis and thus complicate the vitamin C picture. After elimination of these cases, the figures for the 3 groups were as indicated in Table III. No changes were made

in group 1 because no causes of gingivitis were operating effectively in those cases.

Comparing Tables I and III, it is apparent that when the cases known to have local causes of gingivitis are excluded, the values for the vitamin C level in groups 2 and 3 are slightly higher. In other words, the inclusion of the cases having calculus and the like lowered the vitamin C figures for groups 2 and 3. From this again, it may be concluded that a low vitamin C level and general mouth neglect tend to be associated. It is quite probable that both are the result of the generally neglectful and careless habits of the patients.

It was thought that the inclusion of children and adolescents in the groups with adults might have introduced a disturbing factor, but after cases below the age of 20 were taken from the groups the figures were the same. Gingivitis, unlike periodontosis, appears to attack children to much the same extent as it does adults, and in them bears no different relationship to the vitamin C level of the blood plasma.

TABLE III

	NO. CASES	RANGE	VIT. C LEVELS (MG. PER 100 CC. PLASMA)		
			Mean	Median	Mode
Ging. 1	743	0.04–1.92	0.56	0.52	0.40–0.60
Ging. 2 (minus known causes)	288	0.08–2.16	0.516	0.42	0.20–0.40
Ging. 3 (minus known causes)	63	0.08–1.96	0.525	0.40	0.20–0.40

2. *Vitamin C and Periodontal Disease*

In the study of the relationship between the vitamin C content of the blood plasma and the incidence of periodontal disease it was thought best to exclude from the figures the records of all patients under 20 years of age. Below that age it was found that the incidence of the disease was practically zero, and the inclusion of such records might have falsified the whole group. This is in accord with the findings of other workers such as Brekhus (28), who showed that there were practically no extractions for periodontosis up to the age of 20. As with the gingivitis cases, the periodontal cases were graded 1, 2 and 3, indicating respectively no periodontal trouble, moderate, and severe periodontal disease. The ranges in vitamin C level and the means, medians and modes for these groups are shown in Table IV.

When the 3 periodontal groups were subdivided according to the gingival conditions it was found that there were no marked differences in the vitamin C levels. It is interesting to note, however, that the gingivitis and periodontal diseases have a strong association. Table V is added to show this relationship, giving the percentage of each gingival condition to be found within each of the periodontal groups.

If a low vitamin C level were the principal etiologic factor in both gingivitis

and periodontosis, or in cases which combined the two diseases, the vitamin C in cases having both severe periodontal and severe gingival trouble would be lower than in any other group. This was not true. It was found that for the group of such cases, combining both severe gingivitis and severe periodontal disturbance, the mean vitamin C level was 0.499 and the median 0.42. These figures are not appreciably different from the levels of the 2 and 3 gingivitis groups or the 2 and 3 periodontal groups alone. They tend to show only that the neglect of hygiene and the neglect of proper diet are associated, and express only the nature of the person, rather than any direct cause and effect relationship between the diet and the mouth condition.

TABLE IV

	NO. CASES	RANGE	VIT. C LEVELS (MG. PER 100 CC. PLASMA)		
			Mean	Median	Mode
Periodontal 1	533	0.08–2.16	0.559	0.48	0.40–0.60
Periodontal 2	320	0.08–2.04	0.496	0.42	0.20–0.40
Periodontal 3	118	0.08–2.00	0.508	0.40	0.20–0.40

TABLE V

	GINGIVITIS 1	GINGIVITIS 2 AND 3
Periodontal 1	82.5%	17.5%
Periodontal 2	26.2%	73.8%
Periodontal 3	5.0%	95.0%

TABLE VI

	PER CENT CASES ABOVE THE NORM (0.52 MG. VIT. C)	PER CENT CASES BELOW THE NORM (0.52 MG. VIT. C)
No periodontal disease (1)	46.8	53.2
Moderate periodontal disease (2)	40.6	59.4
Severe periodontal disease (3)	39.8	60.2

The median for the cases having neither gingivitis nor periodontal disease was found to be 0.52, the same as that of the group selected on the basis of freedom from gingivitis alone. The percentages of cases above and below this level in each of the periodontal groups are shown in Table VI.

It is to be noted that various gingival conditions are included in each of the above periodontal groups. Less difference is shown between the different groups in the above table than was shown between the groups selected on the basis of gingival condition, as shown in Table II. While the differences between the two sets of figures are not great, they appear to indicate that there is less relationship between the vitamin C level and the periodontal condition, than between the vitamin C level and the gingival condition. This appears reasonable, but not

significant. Both the gingival condition and the vitamin C level can change rapidly, so that one could change along with variations in the other. Periodontal disease, on the other hand, is a more permanent change in the supporting tissues, and, even if caused by a long continued low vitamin C level would tend not to show a comparable degree of repair in response to an increase in the vitamin C level. For this reason these figures do not afford any real basis for considering that either disease is more the result of a dietary deficiency than is the other.

3. *The Effects of Citrus Fruit Intake*

The foregoing data has not included any case in which there was a record of citrus fruit intake within 3½ hours previous to the vitamin C determination. There were 135 such cases excluded. A study of these cases raises interesting questions. The median for this group was 0.64 mg. vitamin C per 100 cc. plasma. The medians for all other groups were between 0.40 and 0.52 mg., with 0.52 accepted as normal. The difference between 0.52 and 0.64 probably indicates the average increase in the vitamin C level caused by the citrus fruits.

It is possible, however, that the citrus fruit records represent, at least to a fair degree, the diet habits of the people from whom the records were obtained. In this group it was found that the percentage incidence of gingivitis was 33.3, while for all groups without citrus fruits the incidence was 44.1. The incidence of periodontal disease among those over 20 years of age who partook of citrus fruits was 43.5 per cent while that among those who did not was 49.9 per cent. This may indicate that the inclusion of adequate citrus fruits in the diet may help to prevent gingivitis and, to a lesser extent, periodontitis, either by supplying vitamin C or in some other way. If this is a proper interpretation, it is in agreement with the findings of Hanke and Hawkins, previously referred to (23–26) which were to the effect that when diet was supplemented with orange juice, gingivitis tended to clear up. On the other hand, it must be pointed out that it might just as well be said that the persons who regularly included citrus fruits in their diet were probably of the type who would try to take care of their mouths, and would therefore be in better condition for that reason.

4. *Seasonal Variations in Vitamin C Level and Gingivitis*

Weisberger, Young and Morse (20) reported that administration of vitamin C produced improvement in the mouth conditions within 2 weeks. From this it would be expected that if vitamin C and gingivitis are linked in a cause and effect relationship, changes in the gingival condition should follow changes in the vitamin C level with a lag of not more than two weeks. It might be assumed that more people would partake of citrus fruit more frequently when the supply was good and the price low. Whether this assumption is correct is problematical, because it was found that the proportion of cases giving record of recent citrus fruit intake was substantially the same for each month or quarter.

The records in this study were collected over the whole of a 2 year period, and the number of cases is great enough to give fairly reliable results, so that a consideration by seasons is justifiable. When the figures for gingivitis are arranged

by quarters, it is found that the percentage incidence of gingivitis is lowest in the winter quarter and highest in the spring quarter. This relationship is shown in *fig. 1.*

A similar variation occurs in the vitamin C levels, as judged by the medians. The vitamin C level is high in the gingivitis-free cases at the same time as the

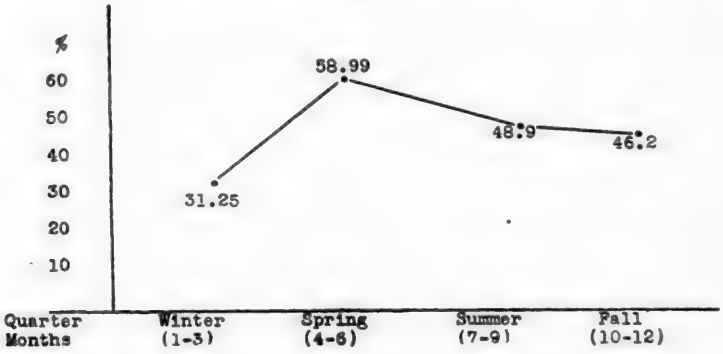

FIG. 1. Percentage incidence of gingivitis by seasons

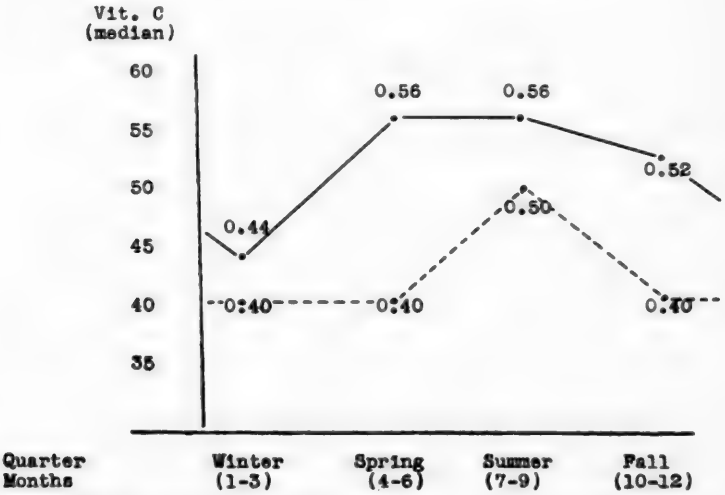

FIG. 2. Seasonal variations in vitamin C level. ——, gingivitis-free cases (1); - - - -, gingivitis cases (2 and 3).

percentage of gingivitis cases is high, but there is no elevation at that time in the vitamin C level of the cases having gingival disease. The seasonal variations in vitamin C level are shown in *fig. 2.*

These figures are for patients as they first entered the clinic and are not repeated observations on the same patients. It will be noted that the incidence of gingivitis (*fig. 1*) was lowest during the winter quarter, when the vitamin C levels

were low for all groups. If a low vitamin C level were the cause of gingivitis it would seem that the incidence of gingivitis should be up when the vitamin C is low. In the group of cases numbered 2 and 3, having gingivitis, the vitamin C is high only during the summer, when the incidence of gingivitis has dropped well below its peak. These figures make it difficult to see a direct relationship between low blood plasma vitamin C and gingivitis.

It may be that the seasonal changes in the computed incidence of gingivitis represent a tendency for the gingivitis patients to come into the clinic more at one time of the year than at others. If this is true, the figures on seasonal incidence do not show the facts. Whether such explanation is correct cannot be learned from the present data, but the possibility must be kept in mind to induce some skepticism of the results.

From *fig. 1*, it will be noted that the low incidence of gingivitis follows the sunshine season. In the summer not only is there more sunshine to generate vitamin D in the body, but also more fruits, green vegetables and salads rich in vitamins A, B, and C are available. Vitamins A and D are said to be stored in the body for long periods. Vitamin C may perhaps be stored for many weeks. The low incidence of gingivitis in the winter quarter, therefore, may coincide with an adequate supply of these vitamins in the body, stored up from summer. In spring, when it may be supposed that the stored up vitamins are being exhausted, the incidence of gingivitis is highest. In summer and fall the body is recovering from the shortage of the spring and is storing up vitamins A and D again, and it is found that the incidence of gingivitis is dropping.

It will be noted in *fig. 2* that the vitamin C level for the gingivitis-free cases was high in the spring. Possibly the supply of citrus fruits is greatest and the prices lowest at this time. The change may mean nothing more than that those people who are inclined to have an adequate diet when possible, and may also be inclined to take care of themselves in other ways as well, are taking advantage of market conditions.

Another possibility is that some people may not absorb the vitamin C properly or may require more of it, so that their blood levels change more slowly than those of their more fortunate brothers, requiring increased intake of citrus fruits over a longer time before any result is shown. A third possibility is that the maintenance of good mouth conditions may require adequate supplies of many food factors, some of which may not be sufficient, or some of which may not be absorbed, in the absence of others. The absence of gingivitis in the experimental scurvy reported by Crandon, Lund and Dill (12) may give support to this idea. The answers to the questions thus raised do not lie in the present data.

SUMMARY AND CONCLUSIONS

Information concerning the gingival and periodontal conditions and the level of vitamin C in the blood plasma of 1396 patients has been collected over a period of two years. The resulting figures have been studied to determine whether there is any causal relationship between low vitamin C levels and the presence of gingivitis and periodontosis.

It was found that the blood plasma vitamin C levels tended to be lower in the cases having gingivitis than in those free from gingivitis. The cases with periodontal disease also tended to show lower vitamin C levels than those without. In proportion to the range or amount of variation in vitamin C levels within each group, however, the differences between groups were very small and are probably not significant. The seasonal variations in vitamin C level and incidence of gingivitis were such that no causal relationship between low vitamin C and the presence of gingivitis was apparent.

These findings suggest that a patient who will neglect his mouth will be likely to neglect his diet, so that gingivitis and periodontosis may arise from the same neglect that allows the vitamin C level to be low. The poor nutritional condition and the poor mouth condition may proceed from the same cause rather than the one from the other.

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RELATIVE ALVEOLOCLASTIC EXPERIENCE OF THE VARIOUS TEETH[1]

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As far as can be determined, an investigation of the precise relative susceptibility to alveoloclasia of the various individual teeth has neither been performed nor reported. On the other hand, a study of the relative caries susceptibility has been published by Klein and Palmer (1). Their work revealed that the various teeth tended to fall into 5 different categories of relative degrees of susceptibility; differences in caries experience between teeth in the same category were explained by the concept of "post-eruptive tooth age."

While other caries investigators did not report the exact differences in caries susceptibility (2), they related the observed varying caries experiences to anatomic features, eg., bulk of crown and shape of occlusal surfaces.

Similarly, an exact knowledge of the way in which the various individual teeth compare with each other in susceptibility to alveoloclasia is essential. This information would enable a choice of those theories of the etiology of alveolar bone destruction which would explain the differences observed. It would seem likely that those theories most tenable would be the more applicable from a general point of view and, therefore, worthy of further study.

PROCEDURE

Roentgenograms of patients selected at random from the Periodontia Clinic were employed for the study. From each patient's chart and roentgenographic series, the following information was recorded: 1, Name, age, sex and occupation, 2, Appearance of the gingival tissues (information obtained from the periodontia work chart), 3, Subjective complaints, systemic disturbances, etc., 4, Enumeration of the missing, unerupted or impacted teeth and the presence of fixed bridgework, 5, The alveoloclastic experience of each remaining erupted tooth.

The procedure for measuring amounts of alveoloclasia for each tooth was adapted from a former study by the authors (3). Each erupted tooth seen in the roentgenographic series was given an alveoloclasia number ranging from 0 to 5. A unit of 0 was ascribed to a particular tooth when there was no bone dissolution evidenced; 5 units were given when the bone was destroyed to the apex of the tooth. A unit of 3 was recorded when the bone destruction had left one-half of the root unsupported. Units of 1, 2 and 4 were estimated as fractions of 3 and 5. Thus, each case had as may alveoloclasia numbers as there were erupted teeth present.

[1] This work was made possible by a grant from the Bristol-Myers Co. Read at the 20th General Meeting of The International Association for Dental Research, New York, March 14 and 15, 1942 (*J. D. Res.*, **21**: 311, 1942). Received for publication March 5, 1942.

RESULTS

A total of 500 cases was studied. There were 307 females to 193 males, corresponding roughly to 3 females to each 2 males. Most individuals were in the age groups of from 30 to 39 and from 40 to 49 years. The mean age was computed to be 39.7 years.

A former study by the authors (3), based on examination of over 1000 roentgenographic series, indicated that males were slightly more susceptible to alveoloclasia. The present investigation confirmed this finding (Table I and *fig. 1*). The males had a mean alveoloclasia experience for all teeth of 21.6 compared with 18.4 for the females. The numerical values represent the average sum of the alveoloclasia numbers per patient. The greatest difference in mean total alveoloclasia values between the sexes was found in the age range 40 to 59 years. There is an apparent drop in mean total alveoloclasia experience in the older age groups. This was attributed to an increased number of missing

TABLE I

Number of cases in each age and sex category and the mean total alveoloclastic experience (B.D.) found in each group

The greatest concentration of cases lay in the age groups of from 30 to 39 and from 40 to 49 years. Males evidenced a greater susceptibility to this factor.

AGE GROUP	MALES		FEMALES		TOTAL	
	No.	B.D.	No.	B.D.	No.	B.D.
10–19	9	5.9	8	1.8	17	4.0
20–29	24	12.4	66	14.6	90	14.3
30–39	53	23.2	92	22.0	145	22.4
40–49	69	24.4	95	19.8	164	21.7
50–59	22	27.5	36	17.6	58	21.4
60+	16	18.5	10	13.2	26	18.4
Total.....	193	21.6	307	18.4	500	19.6

erupted teeth in these individuals, particularly, of those teeth most susceptible to this factor. In addition, patients of advanced age with a number of teeth still present probably included those most resistant to alveolar bone loss.

The possibility that the average amount of alveoloclasia shown by each of the various individual teeth might have been related to the number of such remaining members was considered. With this in mind, the percentage of the various remaining individual teeth was computed (Tables II and III). The percentages varied from 98.8 for the mandibular 6 anteriors to a low of 36 per cent for the maxillary third molars; practically no difference was found between corresponding teeth of both sides. In descending order, the diminishing frequency of appearance of the various individual teeth was found to be: 1. Mandibular first and second incisors and canines. 2. Mandibular first premolars. 3. Maxillary and mandibular second molars. 4. Maxillary first and second premolars and first molars. 5. Mandibular third and first molars. 6. Maxillary third molars.

This enumeration of the various individual teeth according to the frequency with which they were absent was found to be unrelated to a tabulation of their relative susceptibility to alveoloclasia. Comparison of the susceptibility of the

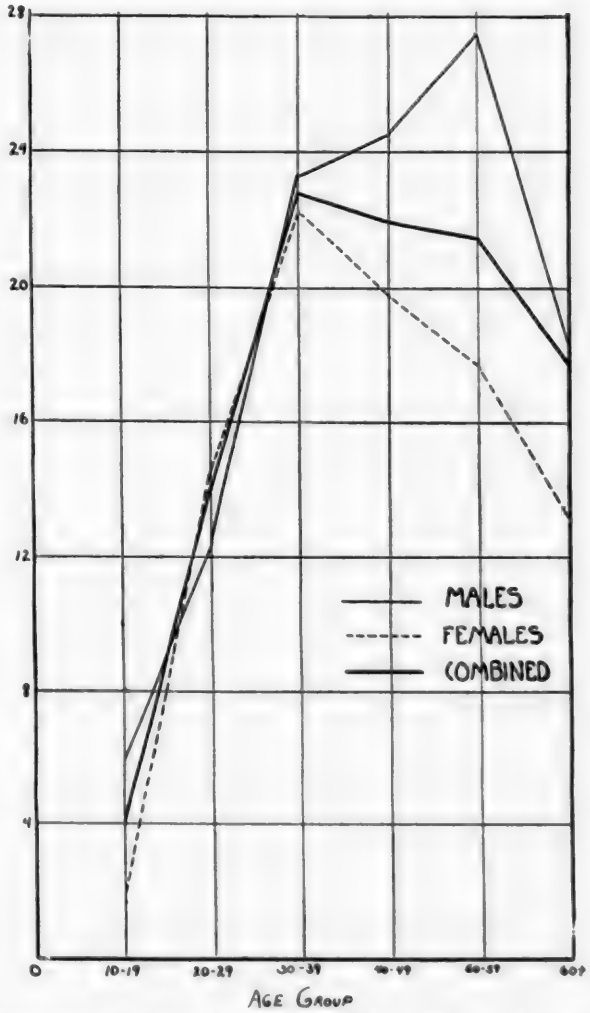

FIG. I. Mean total alveoloclastic experience (see Table I) plotted against age. There is a rapid rise until the ages of from 30 to 39 after which time, the rate of increase is very much reduced in males. In females, after the ages of from 30 to 39, there is an apparent fall in mean total alveoloclastic experience.

various individual teeth was made by computing an alveoloclasia index for each tooth. The index was arrived at by dividing the sum of the alveoloclasia experiences by the number of such remaining erupted teeth (Tables II and IV).

The index varied from .35 for the maxillary left first premolar to 1.37 for the maxillary right first molar. For purposes of evaluation, the teeth were placed into 3 broad categories of relative susceptibility (Table IV).

Group I included the teeth showing the least alveoloclastic experience and contained: 1. Maxillary first premolars. 2. Mandibular first premolars. 3. Mandibular third molars. 4. Mandibular second premolars. 5. Maxillary second premolars. 6. Mandibular canines. 7. Maxillary canines.

The teeth moderately susceptible to alveoloclasia (Group II) were found to be: 8. Mandibular first molars. 9. Mandibular second molars.

The category of greatest order of susceptibility (Group III) was found to include: 10. Maxillary first incisors. 11. Maxillary third molars. 12. Maxillary second incisors. 13. Maxillary left first and second molars. 14. Man-

TABLE II

Data obtained from study of 500 roentgenographic series

Following information is tabulated for each of 32 teeth: number of each of teeth present in entire series (No.); number of each of teeth per 100 cases (% per cent); sum of the alveoloclasia numbers (B.D.) and; a comparative index of susceptibility to alveoloclasia (index) obtained by dividing B.D. by No. Note greater susceptibility of upper teeth compared with corresponding lowers and tendency for teeth on right side to show higher index.

LOWER TEETH					UPPER TEETH				
Tooth	No.	% Per cent	B.D.	Index	Tooth	No.	% Per cent	B.D.	Index
$\overline{8}$	226	45.2	107	.47	$\underline{8}$	191	38.2	180	1.13
$\overline{7}$	354	70.8	326	.92	$\underline{7}$	370	74.0	462	1.25
$\overline{6}$	221	44.2	205	.93	$\underline{6}$	302	60.4	413	1.37
$\overline{5}$	398	79.6	177	.45	$\underline{5}$	348	69.6	203	.58
$\overline{4}$	462	92.4	220	.47	$\underline{4}$	345	69.0	157	.45
$\overline{3}$	493	98.6	262	.53	$\underline{3}$	427	85.4	212	.49
$\overline{2}$	494	98.8	626	1.27	$\underline{2}$	404	80.8	457	1.13
$\overline{1}$	482	96.4	641	1.33	$\underline{1}$	411	82.2	432	1.05
$\overline{1}$	484	96.8	631	1.30	$\underline{1}$	420	84.0	432	1.03
$\overline{2}$	494	98.8	629	1.27	$\underline{2}$	404	80.8	440	1.09
$\overline{3}$	487	97.4	261	.54	$\underline{3}$	421	84.2	205	.49
$\overline{4}$	445	89.0	172	.39	$\underline{4}$	345	69.0	125	.35
$\overline{5}$	379	75.8	176	.46	$\underline{5}$	337	67.4	131	.39
$\overline{6}$	219	43.8	181	.83	$\underline{6}$	280	56.0	326	1.16
$\overline{7}$	350	70.0	296	.85	$\underline{7}$	369	73.8	401	1.10
$\overline{8}$	212	42.4	102	.48	$\underline{8}$	180	36.0	184	1.02
Total.....	6200	77.5	5012	.81		5554	69.4	4760	.86
Grand total.....						11754	73.5	9772	.83

dibular second incisors. 15. Maxillary right first and second molars. 16. Mandibular first incisors.

TABLE III

Tabulation of the various teeth in a descending order of increasing frequency of appearance

Note that the values for the corresponding teeth of both sides are very close to each other

% % RANGE	TOOTH (PALMER INDEX)
35–39	$\underline{8}\vert$; $\vert\underline{8}$
40–44	$\overline{6}\vert$; $\vert\overline{6}$; $\vert\overline{8}$
45–49	$\overline{8}\vert$
50–54	
55–59	$\vert\underline{6}$
60–64	$\underline{6}\vert$
65–69	$\underline{5}\vert$; $\underline{4}\vert$; $\vert\underline{4}$; $\vert\underline{5}$
70–74	$\overline{7}\vert$; $\vert\overline{7}$; $\overline{7}\vert$; $\vert\overline{7}$
75–79	$\overline{5}\vert$; $\vert\overline{5}$
80–84	$\underline{2}\vert$; $\underline{1}\vert$; $\vert\underline{1}$; $\vert\underline{2}$; $\vert\underline{3}$
85–89	$\underline{3}\vert$; $\overline{4}\vert$
90–94	$\vert\overline{4}$
95–99	$\overline{3}\vert$; $\overline{2}\vert$; $\overline{1}\vert$; $\vert\overline{1}$; $\vert\overline{2}$; $\vert\overline{3}$

TABLE IV

Tabulation of the teeth in descending order of increasing alveoloclastic experience

For purposes of analysis, 3 broad categories of relative susceptibility were formed: Group I contained teeth of slight susceptibility, Group II included the moderately susceptibles and, Group III consisted of those teeth which showed a marked alveoloclastic experience.

	B.D. RANGE	TOOTH (PALMER INDEX)
Group I	.29– .38	$\vert\underline{4}$
	.39– .48	$\vert\overline{4}$; $\vert\underline{5}$; $\overline{8}\vert$; $\overline{5}\vert$; $\overline{4}\vert$; $\vert\overline{5}$; $\vert\overline{8}$; $\underline{4}\vert$
	.49– .58	$\underline{3}\vert$; $\vert\underline{3}$; $\overline{3}\vert$; $\vert\overline{3}$; $\underline{5}\vert$
	.59– .68	
Group II	.69– .78	
	.79– .88	$\vert\overline{6}$; $\vert\overline{7}$
	.89– .98	$\overline{7}\vert$; $\overline{6}\vert$
Group III	.99–1.08	$\underline{1}\vert$; $\vert\underline{1}$; $\vert\underline{8}$
	1.09–1.18	$\underline{2}\vert$; $\vert\underline{2}$; $\underline{8}\vert$; $\vert\underline{6}$; $\vert\underline{7}$
	1.19–1.28	$\underline{2}\vert$; $\vert\underline{2}$; $\underline{7}\vert$
	1.29–1.38	$\overline{1}\vert$; $\vert\overline{1}$; $\underline{6}\vert$

The mean combined alveoloclasia indices of the teeth in each of the 3 categories were plotted against age (*fig. 2*). Since the 3 groups follow separate

paths throughout the age ranges of the patients studied, it may be inferred that the relative susceptibility of the various individual teeth are similarly different throughout life.

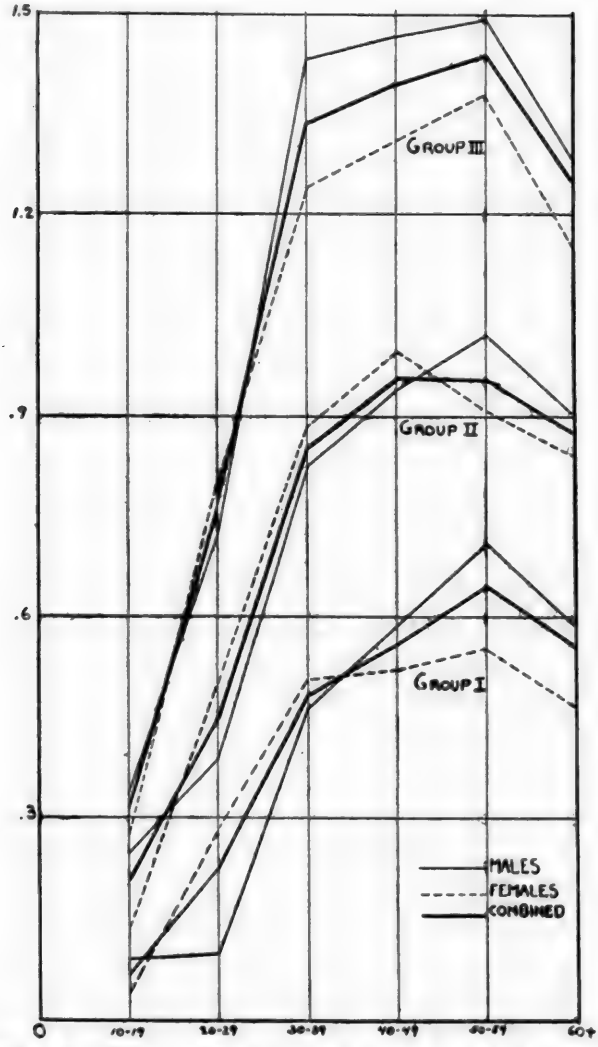

FIG. 2. Mean combined alveoloclasia indices of teeth in each of 3 groups of relative susceptibility plotted against age. Curves for 3 groups do not approximate one another in age ranges of patients included in this study (10 to 72 years).

In addition to the above information, an interesting observation was made. It was noted frequently that where the maxillary first molar was absent unilaterally and all the other maxillary molars were in position, the intact side,

i.e., the side having all 3 molars, showed considerably greater destruction around the maxillary second and third molars compared with the corresponding teeth on the side with the missing member. For example, the cases in which the left upper first molar was absent had a total alveoloclasia experience of the second and third molars of that side of 88 as compared with 112 for the corresponding teeth on the right side where the first molar was still present. This difference in values for the 2 sides was found to be significant statistically. Thus, the absence of the maxillary first molar seems to remove a weakening influence on the bone around the adjoining molars or, simply, no longer permits the spread of alveoloclasia to the approximating teeth.

CONCLUSIONS

The findings demonstrate the well known fact that within the same mouth are seen teeth of varying susceptibility to alveoloclasia. These observed differences will become understandable when a number of properly emphasized varying viewpoints can be correlated. Some conjectures are offered in explanation of the differences which have been pointed out. The eruption time, with its associated growth and metabolic changes, as well as the length of time the tooth is subjected to external forces should be considered. In addition, anatomic features of tooth formation, position and relation probably play a large part in determining susceptibility to alveoloclasia.

SUMMARY

Analysis of roentgenograms of 500 patients who received periodontal treatment revealed that the various permanent teeth tended to fall into 3 broad categories of alveoloclasia-susceptibility. The classes in ascending order of susceptibility were found to contain:

Group I. Maxillary and mandibular first and second premolars and canines and the mandibular third molars.

Group II. Mandibular first and second molars.

Group III. Maxillary and mandibular first and second incisors and maxillary first, second and third molars.

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THE INCIDENCE OF B. FUSIFORMIS AND SPIROCHETES IN THE EDENTULOUS MOUTH[1]

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Dental literature contains the frequently repeated statement that *B. fusiformis* and oral spirochetes are not found in edentulous mouths; that the presence of teeth and the gingival sulcus is necessary for their existence. On the other hand, Disraeli (1) reported numerous cases of Vincent's infection in edentulous children; and a fatal fusospirillary stomatitis in an edentulous adult has been recorded (2). Many instances of noma in infants, (3) who because of their age were presumably edentulous, can be cited. One of us (S. L. R.) has observed an acute fusospirochetal infection immediately following the extraction of all the remaining teeth of a male adult during a subacute exacerbation of Vincent's infection. Fusospirochetal infections of the eyes (4), genitalia (5), extremities (6), lungs (7), and other organs devoid of any structure analogous to the gingival sulcus have been observed.

Despite these contradictory statements, no previous investigation has been made to determine the presence or absence of these microorganisms on the apparently normal edentulous oral mucosa. Investigation of edentulous mouths is to be divided into 4 parts: 1, the healthy edentulous adult; 2, the edentulous adult suffering from systemic disease; 3, the healthy edentulous infant; 4, the edentulous infant with systemic disease. This report concerns the first part, the healthy edentulous adult.

Patients were obtained from the prosthetic clinic of the School of Dentistry (E. H. G.), and from private practice (S. L. R.). Each was examined by direct smear without a prior rinsing of the mouth: 95 were examined by dark field and brightfield smears, the remainder by brightfield only.

Of 68 partially edentulous jaws examined in a preliminary survey, 51 showed the presence of fusospirochetal organisms on the gingivae of the remaining teeth. Of these, 37 (72.5 per cent) showed these organisms on the edentulous space also. Of 20 mouths in which one jaw was completely edentulous, and the other at least partially dentulous and in which spirochetes and fusiform bacilli could be found, 12 (60 per cent) of these had the organisms on the edentulous jaw in equal or lesser numbers. Spirochetes viewed by the dark field were actively motile.

Two hundred and twelve completely edentulous mouths of 92 males and 120

[1] Read at the 20th General Meeting of the International Association for Dental Research, New York, N. Y., March 14–15, 1942 (*J. D. Res.*, **21**: 308, 1942). Received for publication March 28, 1942.

females between 21 and 87 years of age were similarly examined. Their mucosae were intact and there were no visible areas of inflammation. The smears were characterized by the paucity of microorganisms and by difficulty in staining and resembled oral smears from the lower mammalia (8) in which the bacterial cells are also few and scattered. The saliva was mucinous. The salivary pH was determined in only 2 cases and was not alkaline. Spirochetes and fusiform bacilli together were found in 2 instances (0.9 per cent). Spirochetes alone were found 7 times (3.3 per cent). Fusiform bacilli alone were found in 23 cases (10.8 per cent).

Forty-six complete artificial dentures were examined. They were constructed of vulcanite, resin, metal and combinations of these materials and their surfaces varied from hard and dense to porous and cracked. Cleanliness varied from very clean to extremely dirty with much debris. Smears from these dentures did not reveal fusospirochetal organisms.

Twenty-one areas of trauma under complete dentures were similarly examined. The character of the lesion varied from mild hyperemia to ulceration. No spirochetes or fusiform bacilli could be found.

From these observations it appears that where there is a source of fusospirochetal organisms, as in the partially edentulous cases, the bacteria can maintain themselves on the edentulous mucosa. They were never found in greater numbers, however, than at the source. When all the teeth are lost, the number of all microorganisms is markedly reduced. This can be explained in part by the mechanical factor: the action of the tongue, lips and cheeks, and the flow of saliva can dislodge bacterial colonies more easily when they are not protected by a deep gingival sulcus. The changed character of the saliva may also play a part. It is not unlikely that the increased mucin content is one factor in reducing the bacterial count (9). Certainly a comparative study of the saliva in tooth bearing and edentulous mouths should be made.

Oral spirochetes and fusiform bacilli are found on the apparently normal edentulous oral mucosa; and considering the enormous reduction in the numbers of the entire flora, they are found in perhaps the same proportion as in the healthy tooth-containing mouth.

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COMPARATIVE INFECTIVITY FOR GUINEA PIGS OF FUSO-SPIROCHETAL EXUDATES FROM DIFFERENT DISEASES[1]

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Typical fuso-spirochetal organisms are found in several inflammatory diseases of the gingival and periodontal tissues, as well as in a number of extra-oral diseases. In addition to Vincent's infection proper, Kolle (1917) and Kritchevski and Seguin (1918) found them in the exudate from periodontosis; Schroff and Bartels (1928) and D. T. Smith (1932) found them in "dry socket," and other infections following tooth extraction; and Davis and Moorehead (1923) found them in periapical infections. We have seen the fuso-spirochetal flora regularly in these conditions, and also in marginal gingivitis, in localized traumatic gingivitis, and in third molar flap infections. We have not been able to correlate differences in the dark-field or smear picture with differences in clinical symptoms; although, because of the complexity of the flora, it is possible that such differences may be unrecognized. The flora of severe suppurative periodontosis, for example, is sometimes much more varied than that of the other diseases, showing, in addition to the usual fuso-spirochetal organisms, trichomonads and amebae, large flagellated spirilla, more abundant cocci and other bacteria, and larger numbers of leucocytes. All these forms may occur, however, in clinically typical acute Vincent's infection.

D. T. Smith (1932) noted that he was able to induce fuso-spirochetal lesions in guinea pigs with exudates from periodontosis as well as from Vincent's infection proper. We previously reported the production of experimental fuso-spirochetal lesions with exudates from acute Vincent's gingivitis, chronic marginal gingivitis and localized traumatic gingivitis (Rosebury and Foley, 1939). We have since had similar results with exudates from periodontosis and from third molar flap infections. It seems of interest to determine whether any characteristic differences in animal response can be correlated with clinical differences in source of the infecting exudates.

METHODS AND MATERIALS

Heavy suspensions of exudate or gingival scrapings were prepared in 1 cc. of broth in Wassermann tubes in the diagnostic clinic of the dental school.[2] The suspensions were brought to the laboratory, examined under dark field and inocu-

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[2] We wish to thank Dr. D. E. Ziskin and his staff for providing us with these specimens.

lated into guinea pigs within 1 hour. Five-tenths cc. was injected under the plucked skin of the upper inner surface of the thigh, and the area was then traumatized with the injection needle. The infected animals were examined over a period of 1 month. Details of the experimental disease have been given in a previous report (Rosebury and Foley, 1939). A test was considered positive only when all the following criteria were fulfilled: (a) the initial dark-field examination showed numerous spirochetes, fusiform bacilli, and vibrios; (b) within 1 month, generally in 10 days or less, the animal showed either a localized necrotic abscess at the point of injection or a palpable cellulitis extending from that point; (c) foul exudate could be aspirated from the animal lesion with a syringe; and (d) dark-field examination of the animal exudate showed numerous spirochetes, fusiform bacilli and vibrios.

RESULTS AND DISCUSSION

The results are given in Table I. Specimens from a total of 105 clinic patients were examined under dark field during the course of these studies. Of these, 41 specimens were not injected into guinea pigs because too long an interval

TABLE I

Response in the guinea pig to subcutaneous injection of exudates from different oral diseases

CLINICAL DIAGNOSIS	NUMBER OF SPECIMENS	NUMBER INJECTED INTO GUINEA PIGS	POSITIVE RESPONSES		
			Number	Percentage of injected	Percentage of total
Acute Vincent's infection	49	29	17	59	37
Suppurative periodontoclasia (pyorrhea)	42	25	16	64	38
Marginal gingivitis	8	6	5	83	63
Pericoronitis (third molar flap infection)	6	4	2	50	33
Totals	105	64	40	63	38

had been allowed to elapse before examination and inoculation could be done, and the spirochetes had lost motility, or because the dark-field picture was otherwise atypical. The remaining 64 specimens comprised 29 from acute Vincent's infection, 25 from periodontosis, 6 from marginal gingivitis and 4 from third molar flap infections.

Specimens from all four diseases induced typical fuso-spirochetal lesions in guinea pigs. Forty of 64 injected specimens were infective in all, or 63 per cent. The variation in the results for the four diseases is evidently insignificant. Of the specimens injected, positive responses were not obtained more frequently with acute Vincent's gingivitis than with the other diseases. The values in the last column of Table I are likewise sufficiently similar to indicate that selection of samples for injection did not influence the results appreciably; the percentages of successful takes among the total numbers of specimens are not distinctive. The comparatively high percentages for marginal gingivitis may be attributed to chance in view of the small number of samples tested.

These findings are similar to data obtained by the authors in a study of lung abscess. Of 38 specimens of pulmonary exudate obtained by bronchoscope[3] from 15 patients at intervals during the course of treatment, 29 (77 per cent) yielded typical fuso-spirochetal lesions in guinea pigs; and of 38 samples of gingival scrapings taken from the mouths of the same 15 patients at the same times, 26 (68 per cent) were similarly positive in guinea pigs. All these patients had gingival or periodontal disease. It seemed particularly interesting that in 8 of the specimens of pulmonary exudate (from 7 patients) no spirochetes could be found under direct dark-field examination, but guinea pig inoculation was nevertheless followed by typical fuso-spirochetal infection, and spirochetes were demonstrated in the animal exudates. The abundance of leucocytes and cellular debris in these pulmonary exudates often makes demonstration of spirochetes under dark field difficult. These findings suggest that guinea pig inoculation may be more sensitive than direct dark-field examination for detecting spirochetes, and may therefore be useful as a diagnostic method.

The pathological picture induced in guinea pigs by inoculation of material from all the different sources studied followed the pattern previously described by the authors (Rosebury and Foley, 1939) without distinctive differences that could be correlated with source. It seems evident that a group of microorganisms capable of inducing characteristic pathological changes in the guinea pig is associated with several clinically different diseases. According to the data of D. T. Smith (1930, 1932) and of Proske and Sayers (1934) this group of microorganisms—the fuso-spirochetal flora—comprises a small spirochete, a fusiform bacillus, a vibrio and a streptococcus, all anaerobic. All 4 morphological types were found regularly in these studies, both in suspensions of gingival scrapings, in which they form only part of an extremely varied and complex flora, and in the exudates from animal lesions, in which they may appear to predominate, particularly after several passages.

It may be noted that although the potentially pathogenic fuso-spirochetal organisms are present in exudates from these diseases, other potential pathogens may also be present, and may possibly contribute to the respective pictures of disease in man. It is well known that aerobic viridans streptococci, capable under appropriate conditions of inducing subacute endocarditis, are always present in these exudates. Several members of the genus Bacteriodes, such as *B. fragilis* and *B. funduliformis* (Beaver, Henthorne and Macy, 1934; Lemierre, 1936) have been isolated from diseases of the fuso-spirochetal group. Periodontosis pus itself has yielded *B. melaninogenicum* (Burdon, 1928; Weiss, 1937); and recently (Slack, 1942), actinomyces capable of inducing apparently typical actinomycosis in animals. These facts suggest the complexity of the fuso-spirochetal flora.

SUMMARY

Suspensions of gingival scrapings from 64 of 105 clinic patients were injected subcutaneously into the groins of guinea pigs after dark field examination for

[3] These specimens and the accompanying suspensions of gingival scrapings were provided by Dr. A. E. Johnson of the Chest Service, Bellevue Hospital, New York.

the fuso-spirochetal flora. These included patients with acute Vincent's infection, periodontosis, marginal gingivitis and third molar flap infections. Positive responses in the animals were indicated by development, usually within 10 days, of a necrotic abscess or cellulitis, with aspirable foul exudate containing the fusospirochetal flora. Such responses, without gross differences that could be correlated with source of infection, were obtained in 40 instances (63 per cent) and with the suspensions from all 4 oral diseases. No significant differences were found in the frequency of infectivity of suspensions from the 4 diseases. Similar results were obtained with pus taken by bronchoscope from lung abscess cases and with suspensions of gingival scrapings from the mouths of these patients, all of whom had gingival or periodontal disease. These results indicate that fuso-spirochetal exudates from different human sources, irrespective of the clinical differences in the source disease, are all approximately equally pathogenic for guinea pigs by the method of inoculation employed.

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EFFECT OF DRYING ON BACTERIAL COUNTS OF ORAL MUCOUS MEMBRANES[1]

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Under various conditions it has been shown that organisms in the oral cavity vary greatly as to number, type and distribution (1). Numerical variations were found to be greater in different locations of the same mouths than identical locations in different mouths (2). No significant relationship could be established between the number of organisms in the mouth and the state of oral health (3). Studies have been made (4, 5) on the self sterilizing and bacteriostatic properties of mucous membranes in different oral areas and they have been shown to exist to a varying degree dependent on the location in the mouth. Many drugs have been suggested and credited with surface sterilization of oral mucous membranes without regarding natural factors involved in reducing and fixing organisms in localized areas. It seems necessary, therefore, to determine what other contributing factors there may be, besides germicides and antiseptics, which materially aid in destroying and fixing organisms in localized areas of the oral mucosa.

For this investigation comparative numerical studies were made on bacterial colonies obtained from wet and dried mucosa of similar oral areas to determine whether drying plays any role in reducing the number of living organisms in a chosen area.

PROCEDURE

Two oral areas were selected for study: 1, the mucosa above the gingival portion of the external alveolar plates between the canines of the maxillae; 2, the mucosa at the site of the injection for the mandibular nerve near the mandibular foramen. These areas were chosen because it was found that more organisms could be obtained from these areas than from others (excluding the tongue and interdental papillae) and because of accessibility.

For the anterior maxillary area the following method was used: The bacteria were collected by drawing a sterile wire loop across a 3 mm. area of the wet mucosa. A sterile test tube containing 2 cc. of a 0.85 per cent sterile sodium chloride solution was inoculated with this material and poured into a sterile petri dish. Molten glucose agar was added to the petri dish and thoroughly

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mixed with the inoculated saline solution. After hardening at room temperature the inoculated media was incubated for 24 hours and colonies counted in a counting chamber. Immediately after the bacteria were collected from the mucosa with the wire loop the patient was instructed to close his mouth. After the inoculations were made, cotton rolls were placed in the muco-buccal folds on both sides of the patients mouth, the lip raised and the entire area, dried with the blast from a warm air syringe. After drying a second inoculum was obtained by the same method from an area immediately next to the first area used.

This was repeated on 50 patients. Clinically, the mucous membranes of all mouths appeared normal.

For the mandibular foramen area the following method was used: Several small copper rods of uniform diameter (3 mm.) were made, wrapped in paper and autoclaved. The area was first dried with a blast of air, the sterile rod touched to the area and twisted slightly. The contaminated end of the copper rod was introduced into 1 cc. sterile physiological saline solution in a sterile test tube and shaken. The saline solution was poured into a sterile petri dish and melted, sterile, nutrient agar was added. The saline solution was thoroughly mixed with the agar by a gentle swirling motion. This procedure was repeated on the same area after the patient had closed his mouth and swallowed. After incubation at 37°C for 24 to 48 hours colony counts were made and the ratio between those from wet and dry mucous membranes computed. In all 20 patients who were thus studied no disease was apparent on the mucous membranes.

The rods were substituted in the second series to assure uniform size of the areas cultured. Investigation showed that equally reliable results were obtained with dry and moistened instruments used to obtain the inoculum from the dry mucosa.

RESULTS

From the anterior maxillary area the number of colonies on plates made from wet mucosa varied from 4,288 to 105. Eighty per cent of the total wet counts approximated the 4,288 count and 20 per cent of the total wet counts approximated the 105 count. The number of colonies on plates made from the dried mucosa varied from 1152 to 0. Twenty per cent of the total counts approximated 1152 count and 80 per cent of the total counts fell between 400 and 0 counts. The average number of colonies from the wet mucosa was 2599. The average number of colonies from the dried mucosa was 339.

For the mandibular foramen area the colony counts from the dried mucous membranes varied between 23 and 2304 with an average of 811. The colony counts from the wet mucosa varied between 640 and 5568 with an average of 2357.

CONCLUSIONS

From the experimental findings it appears that drying might be one factor in reducing the number of living organisms present on the oral mucosa of the

external alveolar plate above the gingivae between the canines of the maxillae, and at the site of the injection for the mandibular nerve near the mandibular foramen.

The opinions or assertions contained in this article are the private opinions or assertions of the writers, and are not to be construed as official or reflecting the views of the Navy Department or the Naval service at large.—Article 113, U. S. Navy Regulations.

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POTENTIAL ACIDITY AND IMPERMEABILITY OF SPONTANEOUSLY PRECIPITATED SALIVARY MUCIN[1]

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In previous reports numerous experiments were described to show that when fermentation was prevented in the incubated saliva a reducing sugar was formed; but when fermentation was not prevented no sugar could be found due to its fermentation as it was formed.

Simmons (1) stated, "Unfiltered saliva shows a steady rate of acid production. This may be due to the liberation of the acid radicals of the prosthetic group or by fermentation of its carbohydrate components on enzymatic hydrolysis." These acids could not have been formed by salivary enzymes as Sherman (2) wrote, "The monosaccharides are all soluble, crystallizable, diffusible substances, unaffected by digestive enzymes and if not attacked by bacteria in the digestive tract, they are absorbed and enter the blood current unchanged."

The use of thymol to prevent fermentation of the carbohydrate in saliva has been frequently referred to by the author in previous reports. In order to determine what effect, if any, this would have on the diastatic action, saliva, saturated with thymol and incubated for 24 hours at 37°C., was tested with a boiled starch solution and Benedict's solution and it was found that the thymol has no apparent effect upon the enzymes.

Having determined that thymol destroys the acidogenic bacteria (3), without destroying the enzymes, the following experiment offers additional evidence that acid is formed by the hydrolysis and fermentation of the carbohydrate in mucin and that the gelatinous mucin precipitate shows considerable resistance to penetration by the supernatant liquid.

The author's teeth and oral cavity were thoroughly cleansed and 50 cc. of saliva were collected (at 0°C. to retard enzymatic activity and precipitation of the mucin) in a flask containing 2 mgm. of bromcresol purple. One and ½ hours were required to collect the saliva and during that period the flow was stimulated 3 times, for about 3 minutes each, by chewing paraffin. The saliva and indicator were shaken vigorousy every few minutes during collection.

Two 50 cc. centrifuge tubes, the lower ⅔ of which terminated in an inverted cone, were used to confine the mucin precipitate to a smaller area. To the sides of one of these tubes thymol crystals were fused, starting about 1 inch above the apex, and 25 cc. of the saliva-bromcresol purple solution was added and shaken vigorously for several minutes. Twenty-five cc. of saliva containing bromcresol purple were placed in a second tube. The 2 tubes were then placed in the incubator at 37°C.

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The supernatant liquid in the 2 tubes remained purple throughout the experiment. That in the second tube, containing untreated saliva-bromcresol purple solution, became slightly lighter and more reddish in color. Two hours after placing the tubes in the incubator the mucin precipitate, together with epithelial cells, bacteria, etc., had settled to the bottom of the tubes, to the depth of about 13 mm. The precipitate in the first tube remained purple throughout the experiment.

Four hours after placing tube containing the untreated saliva-bromcresol purple solution in the incubator the precipitate was perceptibly lighter, tending toward a yellowish-gray color. The yellow color gradually became more marked, especially near the apex, and about 8 hours after placing in the incubator, attained its maximum, indicating a H-ion concentration of approximately pH 5.4, the pH of the supernatant liquid being near the neutral point. Approximately 22 hours after placing in the incubator the yellow color of the mucin precipitate persisted. The contrast in the color of the mucin precipitate in the tube containing the untreated saliva with that of the thymol treated saliva was so marked as to preclude the possibility of doubt or error, and 16 hours after the maximum color contrast appeared equilibrium between the H-ion concentration of the mucin precipitate and the supernatant liquid had not been reestablished. Twenty-four hours after placing the tubes in the incubator a permanent record of the contrasting colors was made with Kodachrome film.

Duplicating this experiment later, pH determinations were made with the glass electrode on the Beckman meter. It was found extremely difficult to separate the precipitate without contaminating it with the supernatant liquid. Decanting and pipetting were unsuccessful, but partial success was attained by carefully inserting a paraffin-wax disc, which accurately fitted the tube at the junction of the precipitate and supernatant liquid, fastened to the end of a wire and then decanting the supernatant liquid and testing it and the precipitate separately. Some contamination occurred but the supernatant liquid was found to be pH 7.02 and the precipitate pH 6.42, 22 hours after placing in the incubator. The whole fresh saliva was originally pH 7.20.

DISCUSSION

In the tube containing thymol the enzymes had remained active but the acidogenic bacteria had been destroyed and no acid was formed. Many previous tests of incubated saliva containing thymol, showed the presence of sugar with Benedict's solution. While there may be a slight reduction of Benedict's copper solution by proteins or other substances in saliva, if the amylolytic enzymes are destroyed by boiling, preventing the formation of a reducing sugar from the complex carbohydrate in mucin, a marked difference will be found when compared with the same saliva in which the enzymes are active and fermentation prevented.

In the tube containing no thymol crystals hydrolysis and fermentation were permitted to take place and acid was formed. Testing with Benedict's copper solution shows that no reducing sugar is present, or, upon hydrolysis with acid, no reducing sugar will be formed.

The degree of acidity attained in the mucin precipitate of the author's saliva depends upon the concentration of the carbohydrate, which has been shown in a previous report (3) to be influenced by the amount of carbohydrate in the diet.

SUMMARY

In the stagnant saliva the carbohydrate component in the spontaneously precipitated mucin undergoes hydrolysis and fermentation with the formation of acids. The gelatinous mucin precipitate shows marked resistance to penetration by the supernatant liquid with which it is in contact.

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MASTICATORY STRESSES AND THEIR RELATION TO DENTAL CARIES[1]

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Research in gnathodynamics, dormant for some time after the days of G. V. Black, is showing distinct signs of resurrection. Klaffenbach (1), Waugh (2), Brekhus (3), and Brekhus, Armstrong and Simon (4) have recently reported on their experiments in measuring masticatory stresses by means of the gnathodynamometer. In 1936, the author (5) published the results of his experiments with this valuable instrument. Recently, through the courtesy of Dr. L. M. Waugh, I was able to use the gnathodynamometer of the School of Dentistry of the University of Minnesota.

OBJECT AND METHOD

The object of this study was to investigate the relationship, if any, between the degree of development of the masticatory apparatus and the incidence of dental caries; and also the effect of use and exercise upon the strength of the jaws and muscles.

Two hundred individuals, 100 male and 100 female, were carefully selected from among several thousand patients who presented themselves at the dental division of the Health Center of the Workmen's Circle in New York. Each individual was carefully examined for caries, and the caries index recorded. The maximum biting stress was recorded.

The individuals selected possessed natural dentitions with certain similar features, so that a scientific study and comparison could be obtained. All cases in which the normal biting strength could possibly be interfered with by abnormal factors, such as the incomplete muscular development prevalent in very young subjects, or the enfeebled musculature of senility, were eliminated. Only subjects between the ages of 16 and 50 were selected. The average age for males was 27.19 years, and for the 100 females, 24.91 years.

The number of natural teeth in the mouth is a great factor in determining the biting stress of an individual. All the cases selected had a complete or nearly complete complement of teeth. The minimum number of teeth present was 26, not counting third molars. The average number of teeth in each mouth for the males was 28.91; for the females, 28.81.

All cases of advanced periodontoclasia and painful gingival conditions, of deep caries with pulpal involvement, or where the pulp had been devitalized and

[1] Read at the 20th General Meeting of the International Association for Dental Research, New York, March 15, 1942 (*J. D. Res.*, **21**: 319, 1942). Received for publication March 20, 1942.

treated were eliminated. Only individuals with incipient dental caries, or in whom cavities had been eliminated by fillings were included. It was assumed

TABLE I

Male

NAME	AGE	NO. OF TEETH	CARIES INDEX	POUNDS OF PRESSURE		REMARKS
				Left	Right	
B. H.	18	29	30	125	130	
C. O.	39	29	25	75	115	Lost L. L. 6
J. T.	17	26	29	75	88	
I. M.	18	28	23	120	120	
M. W.	16	28	0	125	125	Perfect mouth
N. L.	31	27	15	80	180	Chews on R. side only
H. B.	16	28	17	95	95	
M. B.	19	27	32	85	35	Chews on L. side only
I. G.	43	30	11	115	115	
I. L.	33	26	26	110	85	Lost L. R. 6
I. S.	50	28	30	150	200	
H. C.	46	30	10	60	110	Lost U. L. 7
S. S.	16	28	15	115	115	
N. G.	16	28	19	130	130	
Average	27.19	28.91	18.96	112.86	119.58	

TABLE II

Female

NAME	AGE	NO. OF TEETH	CARIES INDEX	POUNDS OF PRESSURE		REMARKS
				Left	Right	
A. H.	45	29	20	135	135	
M. G.	41	26	44	55	55	
H. P.	37	31	7	90	90	
M. F.	19	28	13	110	110	
A. G.	66	28	28	75	75	
A. R.	21	28	9	85	83	
S. S.	24	32	10	100	100	
F. M.	24	28	31	50	48	Lost U. R. & L. 5
M. I.	35	28	30	50	58	
A. R.	42	28	41	52	55	
K. P.	28	32	0	115	118	
N. D.	19	28	36	68	72	
W. M.	26	30	23	87	88	
C. P.	42	30	13	74	122	Lost L. L. 6
Average	24.91	28.81	23.82	83.91	85.75	

that these factors cannot affect the biting strength or interfere with the free exertion of masticatory stresses.

The mouth piece of the gnathodynamometer was inserted at the first molar

region and the subject was told to bite as firmly and as forcefully as possible to attempt measuring the maximum pressure. In many cases, the test was repeated once or twice, and the maximum registration was recorded. Both sides of the mouth were thus tested.

Tables I and II represent parts of our records of 200 individuals. Column 1 indicates the name; column 2, age; column 3, number of teeth; column 4, caries index; and columns 5 and 6 record, in pounds, the maximum pressure each individual was capable of exerting on the left and right side, respectively. The tables also record the average age, number of teeth, caries index, and pounds of pressure for the male and female patients.

RESULTS AND DISCUSSION

In the male group, the highest masticatory pressure recorded was 210 pounds, and the lowest 55 pounds. Average for the 100 males was 112.86 pounds on the left side, and 119.59 on the right. For females, the highest was 165 pounds and the lowest 25 pounds. Average 83.91 pounds on the left side and 85.75 on the right.

The caries index ranged from zero to 50 in the male group, average 18.96; for females, zero to 51, average 23.82.

A comparison of the male and female records shows that the biting stress of the average male is much greater than that of the average female. On the other hand, the caries index of the average male was lower than that of the female. A similar inverse ratio between the amount of biting stress and the dental caries index seems to exist when comparisons are made within the sex groups. This indicates that the state of development of the masticatory apparatus, as judged from the strength of the bite, is a determining factor in the incidence of dental caries.

The state of development of the masticatory apparatus, in turn, depends chiefly upon the amount of use and exercise to which it is subjected during the process of mastication. This is clearly illustrated in cases of unilateral mastication. Many individuals form the habit of chewing their food on only one side of the mouth, generally due to a painful condition on the other side. In many instances, unilateral mastication remains a habit long after the original cause has been eliminated. In general, individuals with normal bilateral chewing show little or no variation in the masticatory pressures recorded for the right and left sides. However, in cases of unilateral masticatory function, the unexercised side in many cases could exert only half or less the pressure produced by the functioning side.

CONCLUSIONS

1. The state of development of the masticatory apparatus depends greatly upon the degree of function and exercise of the teeth, jaws, and muscles during the process of mastication.

2. There is an inverse proportion between the degree of development of the masticatory apparatus and the incidence of dental caries.

3. Caries can be reduced by vigorous exercise of the teeth in mastication.

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ROLE OF THE DENTAL PAPILLA IN EARLY TOOTH FORMATION

PART 1—ROENTGENOGRAPHIC STUDY[1]

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The interdependence of the enamel, dentin and pulp has long been a subject of experimental investigation [Legros and Magitot (1), Tomes (2), von Brunn (3), Huggins, McCarroll and Dahlberg (4), Hahn (5), and others]. Most of the studies of relations of the component tooth parts deal with the formation and calcification of the tissues at the very earliest stage of development. Relations of the component parts after complete formation of the tooth have also been studied (6, 7, 8, 9). However, very little work has been done, in vivo, to determine the influence of the dental papilla on the newly formed tissues after the formation of the enamel matrix, the latter described by Chase (10) as the acid resistant enamel, and by Diamond and Weinman (11), as the enamel first formed in the organic state which later becomes calcified.

In order to learn more about the role of the dental papilla in tooth development after formation of the enamel but previous to its complete calcification, and also the effect of the papilla upon the growth of bone in the surrounding structures, a technic was devised for the removal of the intact dental papilla, leaving the remaining portions of the tooth undisturbed in the jaw.

The cat was selected for this experimental study because it has been shown previously that the dentition has a growth curve similar to that of man, and completes its cycle of development within a relatively short period of time, about nine months (12, 13).

OPERATIVE TECHNIC AND PROCEDURES

Under ether anesthesia, an incision was made on one side of the mandible through the soft tissues overlying the outer border of the jaw of young cats. The tissues were retracted and a window was cut through the cortical bone with a small surgical bur. The intact dental papilla, including the entire odontoblastic layer, was then removed from the developing permanent canine tooth through its apical region, and the wound sutured. The developing canine tooth of the opposite side of the mandible served as a control in each case. Post operative recovery was uneventful. A diagrammatic drawing of the field of operation is shown in *fig. 1*.

[1] Presented at the 20th General Meeting of the International Association for Dental Research, New York City, March 14th, 1942 (*J. D. Res.* **21**: 301, 1942). Received for publication March 20, 1942.

Intra-oral roentgen examination of the mandible, which included both the operated and control side, was made preceding and immediately following operation, and at intervals thereafter.

OBSERVATIONS

Examination of the roentgen films indicates a number of interesting features. *Fig. 2* consists of 3 roentgenograms of the jaw of one of the experimental animals. The first of these (a), is a picture taken immediately after operation.

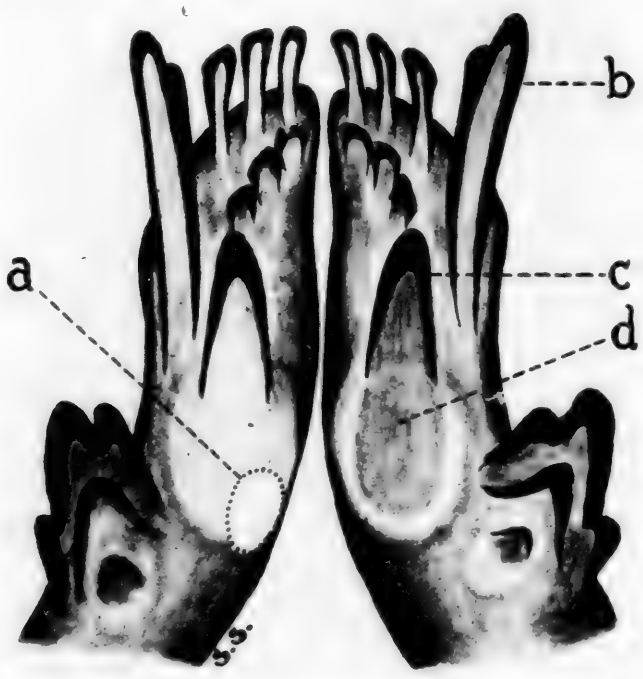

FIG. 1. Diagram from a post-operative roentgenogram taken immediately after removal of the dental papilla from the developing permanent canine tooth on one side of the mandible of the cat. a—window in cortical bone through which papilla was removed, b—deciduous canine tooth, c—calcified portion of developing permanent canine tooth on control side, d—papilla of developing permanent canine tooth on control side.

The second (b), shows the condition 4 weeks later, and the third (c), $3\frac{1}{2}$ months after operation, at which time the animal was sacrificed and the jaw prepared for microscopic study. Examination of the first of these films seems to indicate that the removal of the papilla has resulted in no disturbance of the calcified shell of the developing tooth or its surrounding structures. At this stage, the deciduous canine teeth are shown to be completely calcified and the deciduous incisors show signs of root resorption.

In the last film of this series, $3\frac{1}{2}$ months after operation, the permanent canine

of the control side has erupted, complete calcification of the enamel has occurred, and the root of the tooth is about $\frac{2}{3}$ completed. The permanent incisors on both sides have developed normally. On the operated side the root of the deciduous canine tooth is undergoing resorption although the permanent canine appears to

FIG. 2

FIG. 3

have ceased its growth. This bears out previous experimental work which showed that pressure exerted by the developing permanent tooth plays little part in the resorption of the deciduous root of the antecedent tooth (14). In the area from which the papilla has been removed, the tissue appears more dense in character and a slight irregularity of the outer layer of the enamel can be observed.

Fig. 3 consists of 4 roentgenograms of another of the experimental animals. The first of these (a), is a picture taken immediately after removal of the dental papilla on one side. The second (b), shows the condition 4 weeks later, the third (c), 3 months later and the fourth (d), 7 months after operation, at which time this animal was also sacrificed for histological examination.

In this case, as in that shown in *fig. 2*, a similar progressive picture is shown except that the animal has been permitted to live longer. In the last film of this series the canine tooth of the control side is almost completely calcified whereas the picture of the operated canine shows further progressive changes. The denseness within the area formerly occupied by the dental papilla has increased. Close examination seems to show a bony formation at the apical region, and the irregularity of the enamel contour, which may be an indication of resorption, is more evident than that of the animal sacrificed at an earlier age.

SUMMARY

1. Roentgenograms of the jaws of young cats from which the dental papilla of a developing permanent canine has been removed unilaterally show evidence of a greatly reduced rate of growth of the operated tooth as compared with the control of the opposite side.

2. Close examination of the operated area seems to indicate a rather dense bony formation at the apical region extending somewhat into the area formerly occupied by the papilla, while further within this area soft tissue appears to be present.

3. Resorption of the enamel of the operated side is indicated by an irregularity and a lack of sharpness in detail as contrasted with the control canine of the other side.

These initial observations are limited to roentgenographic examination of the jaws of 2 experimental animals. A histological study in these cases and in additional animals is in progress. In a report of the microscopic findings we are interested in possible effects on the enamel, on calcification, on the dentin, the cementum, and the peridental membrane.

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TOOTH ERUPTION: AXIAL MOVEMENT OF TEETH WITH LIMITED GROWTH[1]

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INTRODUCTION

Our knowledge of tooth eruption is still in a hypothetical stage. It was felt that only a more detailed and minute histological investigation of erupting teeth could possibly lead to an understanding of this complicated phase in tooth development.

It seemed promising to attack this problem by: first, investigating specialized teeth in which the eruption is, in a sense, simplified; such teeth are the continuously growing and erupting teeth. Second, only one component of the complicated movement of erupting teeth should be studied; the most obvious movement is the axial movement. For this reason the first study on eruption dealt with the axial movement of continuously growing teeth only (6).

The purpose of this second paper on eruption is to study the axial movement of teeth with limited growth.

REVIEW OF LITERATURE

In a recent paper Schour and Massler (5) gave a critical review of the different theories of eruption. It seems, therefore, unnecessary to repeat a complete account of the literature.

The most important phase in the eruption of teeth with limited growth coincides with root growth as well as growth of the alveolar bone. Either one or both of these processes have been described as causal factors in tooth eruption. Unfortunately, all these theories are expressed in rather general terms and were open to objections, a fact which caused the introduction of new and different theories.

In studies of detailed histological findings of the growing root and surrounding structures, Orban's (2) description of the epithelial sheath at the growing end must be mentioned. He concluded from his observations that the root does not grow into the alveolus but out of it; the epithelial infolding is the fixed point.

The formation of bone in the alveolar fundus during eruption has been studied extensively by Kronfeld (1). The last and most accurate account has been given by Weinmann (7). He was able to show that the apposition of alveolar bone occurs in 2 ways. Bone is either deposited layer upon layer, or new trabeculae are formed at some distance from the older ones. In the first case the rate of growth is slow, in the latter quite rapid. Weinmann uses these differences to subdivide the eruption period into 3 phases. The first includes the movements

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[2] The author of "Tooth eruption: The axial movement of continuously growing teeth" (J. D. Res., **21**: 201, 1942) is erroneously given as Dr. Henry Sicher. The author of that paper and this one is Dr. Harry Sicher.

of the tooth germ preparatory to the eruption in a stricter sense and is characterized by slow apposition of bone. The second phase coincides largely with the development of the root and shows rapid bone formation. The third, starting at the time when the root is completely formed, lasts throughout the life of a tooth and is again a phase of slow bone apposition. Weinmann did not enter into a discussion of whether apposition of bone is a causal or only an accidental factor in tooth eruption.

The descriptions of the periapical tissue of erupting human teeth are, at the best, rather sketchy. It is said to be a loose connective tissue which is well vascularized.

Sicher (6) attempted to find the causes for axial movement of continuously growing and erupting teeth (*fig. 1*). He could show that the pulpal growth alone is responsible for the eruption of such teeth. The pulp grows in a restricted proliferation zone at the basal end just above the infolding of the epithelial sheath (epithelial diaphragm). This growth is simultaneous and equal to the growth of the epithelial sheath itself at the region of the infolding. This pulpal growth is directed in its effect by a "hammock ligament" anchored in the bone above the alveolar fundus and supporting the basal end of the tooth. This ligament protects the bone at the fundus from resorption and allows only an outward growth of the tooth.

PURPOSE OF INVESTIGATION

The investigation on teeth with limited growth had as its first aim to find out whether, during the period of root development, the axial movement is caused and brought about by processes and structures found on continuously growing teeth. It was clear that root growth alone could not possibly account for the eruption of such teeth. First, it is a well established fact that human teeth do erupt after the full development of the root. Second, it is known that, during eruption, the crowns of some teeth travel a greater distance than could be accounted for by the length of the developing root. In other words: some teeth do not only grow out of the jaw but move bodily in the direction of their long axis. Third, the erupting tooth moves in directions which are at angles to the growth of the root.

The attempt, then, had to be made to establish the action of additional "forces" of eruption; or more specifically, to find out whether and in which way the changes in the alveolar bone are causally linked with tooth eruption.

MATERIAL

The material consisted of serial sections of human jaws of different ages. Jaws of dogs and pigs were used to control the findings on human material.

OBSERVATIONS

The histological findings on growing and erupting human teeth differ to a remarkable degree on teeth with 1 or 2, or more, roots. The findings on erupting single-rooted teeth will be described first. They are illustrated on a lower canine and a lower second molar of a child 8 years of age. The canine is sectioned labio-lingually, the molar mesio-distally (*figs. 2* to *6*).

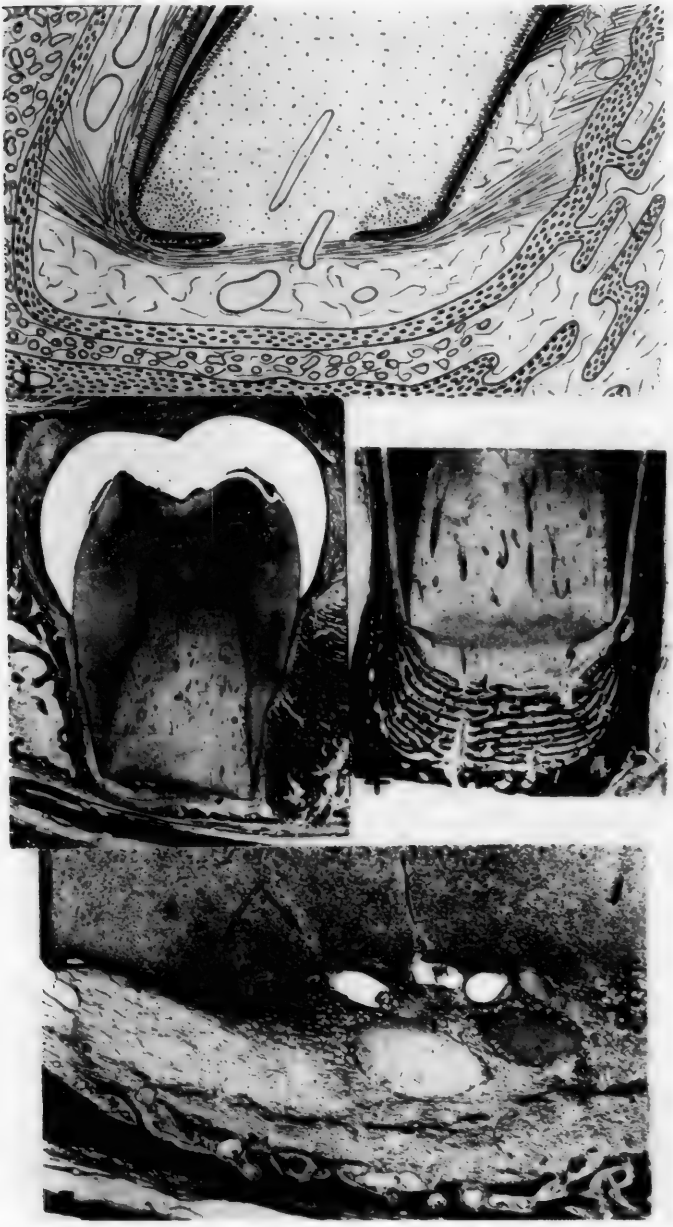

FIG. 1. Diagram of basal end of rat incisor

FIG. 2. Central mesio-distal section through a lower second molar of a child 8 years of age

FIG. 3. Higher magnification of apical region of the tooth of *fig. 2*, distal part. Spaces in cushioned hammock ligament; 2 large spaces filled with coagulated fluid.

FIG. 4. Central labio-lingual section through a lower canine of a child 8 years of age Extensive trabecular bone formation at fundus.

The epithelial sheath of a growing root is, at its basal end, reflected into a plane approximately at right angle to the long axis of the root. It consists of a double layer of epithelial cells, the inner and outer enamel epithelium.

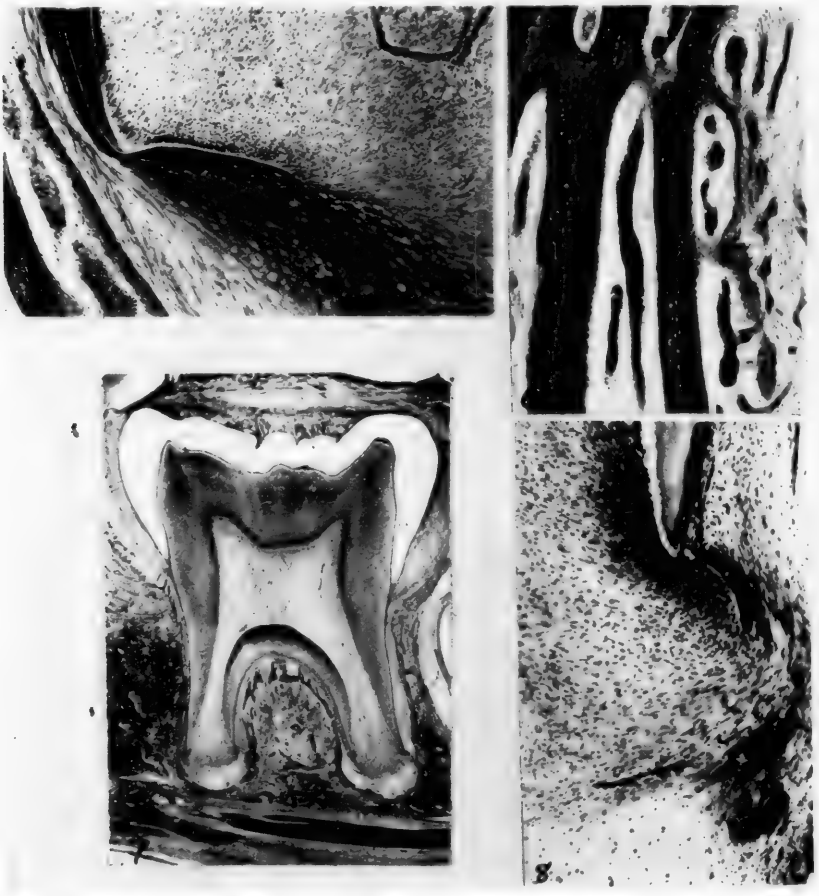

FIG. 5. Higher magnification of part of *fig. 4*. Spaces in hammock ligament; proliferation zone in pulp above epithelial infolding.

FIG. 6. Tooth of *figs. 4, 5;* formation of a new trabecula by multiple bony sprouts

FIG. 7. Central mesio-distal section through lower first molar of a child 4½ years of age. Mesial surface to left. Mesial periodontal membrane narrow, distal wide.

FIG. 8. Distal wall of distal pulp of tooth of *fig. 7* in higher magnification. Epithelial sheath bent distally.

The pulp is clearly limited against the periapical tissue in the plane of the epithelial "diaphragm." The separating line is almost a straight one. The pulp shows at its end a zone of increased density which corresponds to the opening in the epithelial diaphragm. This increase in density is due to a greater number of fibers in this area. Adjacent to the epithelial folds the pulp shows

clearly a proliferation zone (*fig. 5*) where the cells are densely arranged, the fibers very thin and delicate. These fibers are all argyrophile and precollagenous.

The periapical tissue shows a clear division into 2 parts. One zone adjacent to the bony alveolar wall consists of loose connective tissue. It contains many blood vessels most of which, however, are going to pulp or periodontal membrane. The layer adjacent to the growing end of the tooth is of quite different character. It is a tissue which contains large amounts of fluid in spaces between a network of rather thick fibers. These spaces are usually of equal size; in some cases much larger spaces are found, formed perhaps by confluence of smaller. The larger spaces contain, in prepared sections, a coagulated fluid which stains only faintly with eosin. This tissue is of a type found nowhere else in the human body. It may be classified tentatively as related to mucoid connective tissue. Into this tissue, from the periphery, enter strong strands of fibers which are seen to arise in the periodontal area at the side of the root. They curve as a rather strong ligament around the edge of the root and then split up into the network containing the fluid filled spaces. The connection of these fibers with those of the periodontal membrane gives to them a strong anchorage in the bone. This whole apparatus can be designated as a "cushioned hammock ligament."

The bone at the alveolar fundus is growing by trabecular apposition in the canine as well as in the molar. In the latter (*figs. 2* to *3*) the amount of newly formed bone is small. Only a thin layer of bone separates the alveolus from the mandibular canal. The bone is trabecular. The apposition starts irregularly in many places at approximately the same time. Spicules of bone are developing at right angles to the surface and widen out at a short distance from the old bone trabecules. In some places these mushrooming bone growths can be seen approaching each other forming, in this way, trabeculae parallel to the old ones. There is evidence that the old trabeculae are still growing by apposition on their alveolar surface.

At the alveolar fundus of the canine, new bone was laid down in the same way (*figs. 4–6*); formation of parallel trabeculae from short bone spicules, widening out at some distance from the old trabecula and eventually forming a new trabecula, can be observed readily (*fig. 6*). These sprouts shoot up irregularly in many places at the same time. Connecting links between all the old trabeculae give proof for the formation of the whole trabecular bone in the same way. The most striking difference between the alveolar bone of the molar and canine is the enormous increase in the amount of bone laid down in the alveolar fundus of the canine.

The histological investigation of a birooted first molar of a child 4½ years of age gives a strikingly different picture. This tooth (*figs. 7–8*) is approximately in the same developmental stage as the single-rooted second molar described and illustrated. The root is bifurcated and a wide interradicular septum has been formed. The tooth is migrating mesially as evidenced by the narrow mesial and wide distal periodontal membrane. The epithelial sheath at the root ends is short. It is not reflected but continues at the mesial circumference of both roots straight in the direction of the long axis of the roots. At their distal

circumferences it is bent distally, away from the apical foramen (*fig. 7*). The pulp bulges out of the root into the periapical tissue and is bounded by an apically convex line. There is no restricted proliferation zone; the whole protruding part of the pulp shows instead a dense arrangement of nuclei proving it to be the site of growth.

The periapical area, filled with loose connective tissue, is very wide. This tissue shows no signs of any differentiation. Not even a vestige of a "cushioned hammock ligament" can be found.

The bone at the alveolar fundus is at rest. A strongly marked resting line can be seen on its alveolar surface. Only at the crest of the interradicular septum the proliferation of bone is in progress (*fig. 7*). Long and rather regular bony trabeculae grow out parallel to the long axis of the tooth from the last surface layer. At the free ends of the trabeculae new bone is being formed.

DISCUSSION AND CONCLUSIONS

The most significant finding is that of the cushioned hammock ligament. We are justified in explaining structural details of connective tissue in a mechanical sense. And in this light this ligament can serve 2 functions. It is, on the one side, a supporting structure for the growing root end of the tooth. It is, as in the rat, the structure changing this growth into an outward movement. As long as this ligament exists, the tooth cannot grow into the alveolus but has to grow out of the jaw. Of all the processes contributing to the elongation of the root, the growth of the pulp in the proliferation zone adjacent to the epithelial diaphragm is the mechanically important factor in eruption.

That the infolding of the epithelial diaphragm in human teeth is far smaller than in the rat incisor, and that the proliferation zone of the pulp is less developed can be explained by the much slower rate of root growth and eruption in man. Elongation of 20 mm. in the rat incisor takes place in less than 3 months, in man the formation of a root 20 mm. long takes more than 3 years.

The cushioned hammock ligament in human teeth has a second important function. The bone growth at the alveolar fundus is initiated by the development of multiple bony spicules which later spread in a surface plane to form new trabeculae. The pressure of these outgrowths is, therefore, irregular, and the ligament extremely rich in fluid is able to act as an equalizer according to hydrostatic laws. Thus the basal end of the tooth is always under equal pressure however irregular the centers of growth are. It is the proliferation of osteogenic connective tissue, preceding the deposition of bone, which can be viewed as producing the pressure. This should not be considered as a gross mechanical pressure, but rather as a biomechanical stimulus disturbing the equilibrium around the tooth and eliciting reactions in the surrounding tissues which result in reestablishment of this equilibrium. The cushioned hammock ligament is the structure which correlates root growth and bone growth to one directed effect: the axial eruption movement of the tooth.

The findings on birooted teeth, as described on a first molar, furnish conclusive evidence for the correctness of the above explanation. This tooth is quite

evidently under "pressure" of growing bone in the region of the bifurcation. This growing bone alone could easily account for the eruption movement in a longitudinal direction. Thus, the whole tooth would be lifted out of its socket and the roots could grow into the alveolus from the fundus from which they are passively moved away. The histological details of these root ends and their surroundings are in absolute accordance with such an assumption: There is no bone apposition at the alveolar fundus. There is no hammock ligament. The pulp protrudes out of the root end. The epithelial sheath behaves as a passive structure; it is even shaped by the simultaneous mesial drift of this tooth as shown by the different appearance on the mesial and distal sides of both roots. Here, to put it in other words, none of the complicated structures is necessary and none is found which has as its function coordination of root growth and apposition of bone behind the growing root. This in turn seems to be proof for the function of the hammock ligament as explained above.

The last observation also leads to the understanding of the movement of teeth, the roots of which are already completely developed. That the alveolar fundus of such teeth shows continuous bone apposition is well known. Now it can be definitely stated that this bone growth (or better the preceding proliferation of the osteogenic tissue) is the factor causing eruption of these teeth. If root growth and bone growth are the causes of eruption, then the distance which the crown of a tooth travels during eruption in the direction of its axis is, of course, equal to root apposition and bone apposition. Our findings are in accordance with the well known fact that the crowns of some teeth, during eruption, travel to an extent almost equal to the length of the root alone, in other cases a much greater distance. Lower molars, for instance, which develop in close proximity to the mandibular canal are, even after eruption, not very far from this structure; the mandibular canine, on the other hand, developing close to the inferior border of the mandible, travels a distance much longer than its root length until it reaches the occlusal plane. Study of the alveolar fundus of the canine (*fig. 4*) and a second molar (*fig. 2*) shows that this difference is fully explained by the difference in the amount of trabecular bone deposited in the alveolar fundus.

SUMMARY

It could be shown that root growth and bone growth are the 2 factors responsible for the axial movement of erupting human teeth. More specifically, it is the growth of the pulp in a restricted proliferation zone at the apical end of the tooth above the inflected epithelial sheath, and the proliferation of the osteogenic tissue preceding bone apposition which combine to supply the "force" of eruption.

The root ends of single-rooted teeth rest upon cushioned hammock ligaments anchored in bone and periodontal membrane. This ligament consists of a network of strong fibers and large amounts of fluid contained in round spaces between the fibers. It supports the growing root end and directs its growth which becomes the one component of axial movement. It acts at the same time as an equalizer for the "pressure" exerted by the growing bone at the alveolar

fundus. The bone grows by the formation of concentric bone trabeculae; the formation of a new trabecula is initiated by irregular proliferation of short bony sprouts from the surface of the old bone trabecula. The cushioned hammock ligament correlates root (pulpal) growth and bone (osteogenic tissue) growth to one coordinated "force" of eruption.

The amount of bone formed behind the moving tooth is different in different teeth and is proportional to the distance a tooth travels during Weinmann's second phase of eruption.

After establishment of the bifurcation and during the later development of the roots, in multirooted teeth, bone grows only at the crest of the interradicular septum. Thus, the tooth is lifted upward in its alveolus, and the root grows simultaneously into the periapical area. In such teeth not one of the complicated structures which correlate bone and root growth in single-rooted teeth can be seen.

After the root is fully developed, bone apposition at the alveolar fundus is the "force" of eruption.

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THE EPITHELIAL SHEATH: HISTOGENESIS AND FUNCTION[1]

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Epithelium is the primary formative tissue of the human tooth. Starting with the stratum germinativum, the epithelium blazes a trail through the connective tissue, organizing itself and the enclosed connective tissue as it outlines the pattern for a particular tooth. It is our purpose to demonstrate that, in a broad sense, this organization of and by the epithelium takes place in 2 individual units—one of which forms the crown, the other, the root of the tooth. The second unit does not begin to function until the first has virtually completed its functioning.

The familiar arrangement of the 4 epithelial layers which constitute the enamel organ serves to complete the formation of the hard tissues of the crown of the tooth up to the cervical line, or the cemento-enamel junction. The epithelial sheath serves to outline the form of the root, and further functions to organize the adjacent connective tissue for the formation of odontoblasts and root dentin. The capacity of the epithelial sheath to stimulate root dentin is not manifested until the enamel is completely formed and calcification is well on the way. During the interval of calcification of the enamel matrix, dentin continues its formation within the crown portion of the tooth.

REVIEW OF THE LITERATURE

The use of the term "epithelial sheath" was first suggested by Hertwig (5). His observation, made in association with his study of a frog tooth, is recorded as follows: "The lower part of the epithelial mantle can in this instance no longer be designated as the enamel membrane, but one must instead, differentiate it with the term epithelial sheath." This statement in itself is ambiguous, since it is not clear just what was meant by the "lower part." Hertwig, however, continued to state that, "As soon as the crown is almost completely formed, the root of the tooth begins to develop." For root, Hertwig used an uncommon term, "Zahnsockel," which literally means the base of the tooth; but the term apparently has the same connotation as "Zahnwurzel," or tooth root. Since his study was with amphibians, in which the division between crown and root is not clearly defined, the use of the term "Zahnsockel" is somewhat more understandable.

If these 2 consecutive statements of Hertwig are related, it becomes somewhat more clear what Hertwig may have had in mind by the "lower part," and it may be interpreted that Hertwig intended the use of the term "epithelial sheath" for that part of the epithelium which is concerned with the formation of the root portion of the tooth. Hertwig closed his remarks on the epithelial sheath with

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this statement, "By an increase in the length of the epithelial sheath in a downward direction, the tooth outline is lengthened, and the germ tissue as a result assumes the form of the future root." Again Hertwig used the term "Zahnsockel." This statement is quite clear. It is interesting that Hertwig never again referred to the presence of an epithelial sheath and omitted it entirely in his presentation of the development of the human tooth.

Kolliker. Kolliker (6), independently of Hertwig, made the following observation: "When the crown is almost completed and the tooth is about ready for eruption, the tooth germ increases in length, the enamel organ atrophies, and only dentin is formed in the newly forming root." Kolliker made no mention of an epithelial sheath, but evidently disassociated the enamel organ from any participation in the formation of the root.

Von Brunn. Von Brunn (13, 14) took particular note of both Kolliker's and Hertwig's observations. He evidently interpreted them as being in total disagreement. Although Hertwig did not actually make such a statement, von Brunn interpreted Hertwig's statement as implying that the epithelial sheath was a continuation of the enamel organ. Von Brunn stated, "The enamel organ extends not only to the cemento-enamel junction, but way beyond, until the root end is finished."

His evidence for the histogenesis of the epithelial sheath is inadequate, and he utilized another phenomenon to support his contention. Von Brunn's investigations were carried on in part with a rat whose molar teeth present localized enamel-free occlusal areas. He argued that the epithelium covering the localized enamel-free occlusal areas is part of the original enamel organ and consists of inner and outer enamel epithelium in close contact. Yet this part of the inner enamel epithelium of the enamel organ produces no enamel, but activates the formation of dentin only. By analogy, von Brunn maintained that the epithelium, activating the formation of dentin for the root of the tooth, must be of the same nature. To make his analogy more forceful, von Brunn referred to the localized enamel-free occlusal areas as root areas. Von Brunn was unsuccessful in demonstrating the presence of an epithelial sheath in a developing human tooth; in attempting to do this he used a badly macerated specimen selected at random. He was convinced, however, that an epithelial sheath also existed with the human tooth.

Von Brunn summed up by suggesting that all the epithelium concerned with the development of the tooth from the beginning of the tooth bud formation, through the completion of the entire tooth, be designated as the epithelial sheath. Enamel formation, he added, is only an accessory function of the epithelial sheath.

Other Observers. Von Ebner (15), in the study of the deciduous teeth of a 3½ year old human, was unable to find an epithelial sheath. Carl Rose (12), disputed von Ebner and supported von Brunn, contending that the epithelial sheath composed of inner and outer enamel epithelium also existed with human teeth.

Contemporary observers (3, 4, 9, 10), with one exception (7), designated the

region of transitional continuity between the outer and inner enamel epithelium of a human tooth germ as "Hertwig's epithelial sheath" (*fig. 1*). That designation has been perpetuated to the present day. Diamond and Weinmann (2) maintained that this region of transitional continuity between the outer and inner epithelium of the enamel organ represents the future cervical region, or cemento-enamel junction of the tooth, and should be designated rather as the cervical loop.

Mummery (8) took exception to the views of von Brunn (14) regarding the histogenesis of the epithelial sheath. In studying the human tooth Mummery observed,

"It is seen that the enamel organ is not prolonged beyond the point where the follicle is attached to the tooth at its neck, but that the enamel organ terminates where the enamel terminates." . . . "We thus see that the sheath of Hertwig is not a downward extension of the cells of the enamel organ, and we have to look elsewhere for its origin." . . . "With regard to the actual sources of these cells, they are probably derived from proliferations of the tooth band within the follicle, and possibly also from the buddings or proliferations of the external epithelium of the enamel organ.

"It has thus been shown that the enamel organ proper is not continued downwards to form the Hertwig's sheath, that the enamel organ terminates at the point of termination of the enamel, and that the sheath is a prolongation of other epithelial cells in the follicle which lie to its outer side. It is shown that in man, as in other Mammalia, there is every evidence that it moulds and determines the dentin of the root, and is always present where dentin is laid down.

"As it is shown that the sheath is not the continuation of the enamel organ, it may be considered that two separate epithelial organs are formed from the tooth-band and the cells derived from it—the enamel organ, which is especially differentiated to form enamel, and the epithelial sheath, which is the form-determining organ of the dentin."

MATERIALS FOR STUDY, AND PROCEDURE

Since the enamel organ, inclusive of the cervical loop, persists during the period of formation of the hard tissues of the crown of the tooth, it was our desire to find human material at a stage of development when the first evidence of root formation could be observed. Our own collection of human material is almost entirely prenatal and was therefore found inadequate for the purposes of this investigation, because in all of it the hard tissues of the crowns were still in the process of formation. The enamel organ, inclusive of the cervical loop, was still evident; but no indication could be found of the beginning of root formation or evidence of the presence of the formative organ for the formation of the future root.

Fortunately, we were presented by Dr. Gottlieb with a collection of prepared specimens, in serial sections, of human teeth which were in considerably more advanced stages of formation. Even in this collection, however, very few specimens could be found which showed early stages of root formation, and none which showed the critical period of our special interest, the period of the very beginning of root formation. With the cooperation of Dr. Weinmann from the Chicago Foundation for Dental Research, and Dr. Schour from the University of Illinois Dental School, we were able to obtain additional prepared material from their respective collections which was closer to our objective. All of the material was stained with hematoxylin and eosin.

The specific regions studied were the cervical parts of the crown, with the adjacent epithelial tissue, as well as the entire extent of root already formed, with its peripheral epithelial tissue, as far as its deepest terminal end. Photomicrographs under oil immersion were taken of the entire areas indicated; these were accurately related, and mounted. This procedure made it possible to observe the cellular arrangements of the epithelium, and the adjacent connective tissue, in continuity.

FINDINGS

Fig. 1, photomicrograph of a germ of a human mandibular deciduous first incisor, 6 months prenatal, shows evidence of the beginning of dentin formation, and enamel matrix formation in the region of the incisal tip. The enamel organ is intact. The cervical loop regions of the enamel organ are generally designated as Hertwig's epithelial sheath.

Fig. 2, a photomicrograph of a buccolingual section of a mandibular deciduous human molar tooth, 9 months post-natal, shows the state of advanced formation of dentin and enamel matrix. The enamel organ appears generally reduced, but is seen more clearly intact in the cervical region enclosed within square #1. The area enclosed within square #1 is presented under higher magnification in *fig. 3*. The enamel organ in this region is still intact. The functioning ameloblasts may be seen at 'A'. At the terminal end of hard tissue formation, only predentin may be seen. The ameloblasts in this region are not as yet functionally differentiated to produce enamel. The ameloblasts beyond the terminal end of the predentin appear considerably elongated; and if they are followed in the direction of 'A²' they may be seen in an undifferentiated state. The peripheral cells of the connective tissue adjacent to the epithelial cells are undifferentiated, with no evidence of odontoblasts. The enamel organ may be followed clearly to a region at square #1. It may also be observed that the enamel organ narrows considerably as it reaches its terminal point at the end of square #1. The outer enamel epithelial cells gradually become closer to the ameloblasts, seemingly merging with the ameloblasts at the end of square #1. The area enclosed within square #1 is presented in higher magnification in *fig. 4*, the area enclosed within square #2 is presented in higher magnification in *fig. 5* and the area enclosed within square #3 is presented in higher magnification in *fig. 6*.

Fig. 4 shows the zone of stellate reticulum narrowing down, the outer enamel epithelium converging toward the stratum intermedium, and the inner enamel epithelial cells at 'L'. *Fig. 5* shows the cervical loop at the region of continuity between the outer and inner enamel epithelial cells (L). The epithelial layer of cells may be followed to the cervical loop, and beyond. No essential differences in the morphology of the epithelial cells on the enamel organ side of the cervical loop can be seen from the continuity of epithelial cells beyond the region of the cervical loop. The outer enamel epithelial cells, beyond the region of the cervical loop, lie adjacent to the epithelial cells in the position formerly held by the stratum intermedium cells. The peripheral cells of the adjacent underlying

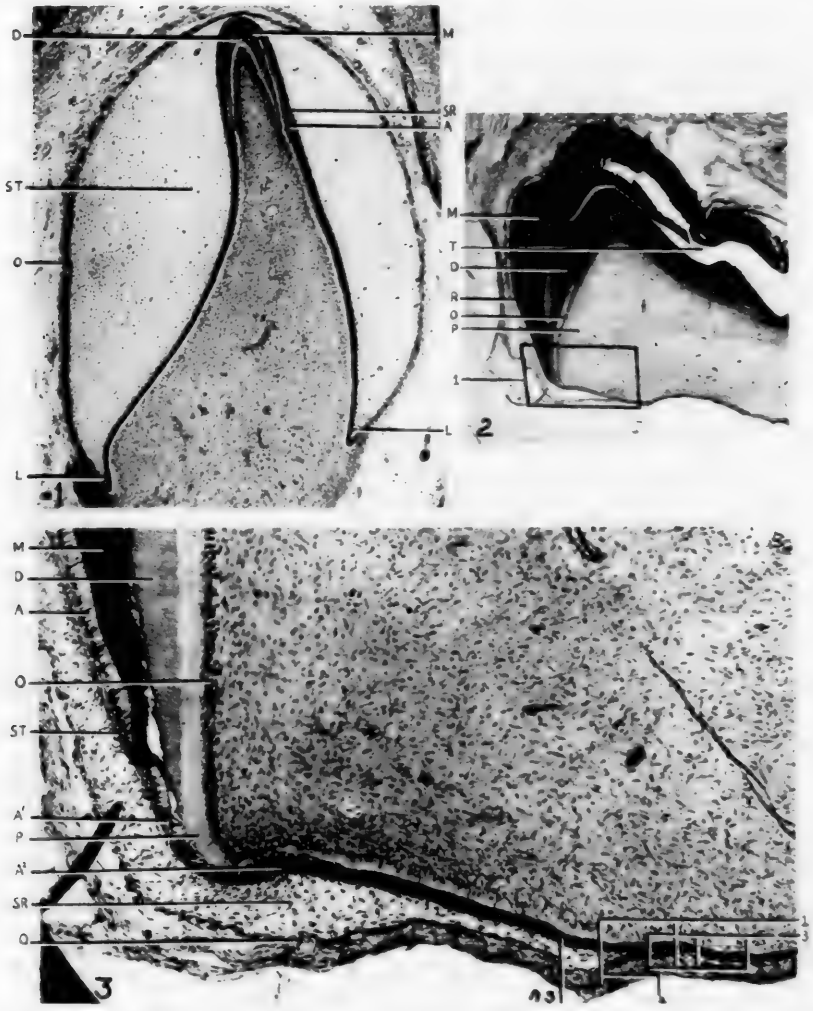

FIG. 1. Labiolingual section of human deciduous mandibular first incisor about 6 months prenatal. M—enamel matrix, D—dentin, ST—stellate reticulum, SR—stratum intermedium, A—ameloblasts, O—outer enamel epithelium, L—cervical loop (usually designated as Hertwig's epithelial sheath).

FIG. 2. Buccolingual section of a deciduous molar developing tooth of a 9 month old human. M—enamel matrix, D—dentin, T—artifact, R—reduced enamel organ, O—odontoblasts, P—pulp. Area enclosed within square *1 is presented in higher magnification in *fig. 3*.

FIG. 3. Higher magnification of area enclosed within square *1 of *fig. 2*. M—enamel matrix, D—dentin, A—ameloblasts, OD—odontoblasts, ST—stratum intermedium, A^1—ameloblasts just prior to state of complete functional differentiation, P—predentin, A^2—elongated ameloblasts, SR—stellate reticulum, O—outer enamel epithelium, A^3—undifferentiated ameloblasts. Areas enclosed within squares *1, *2, *3, are presented in higher magnification in *figs. 4, 5, 6*, respectively.

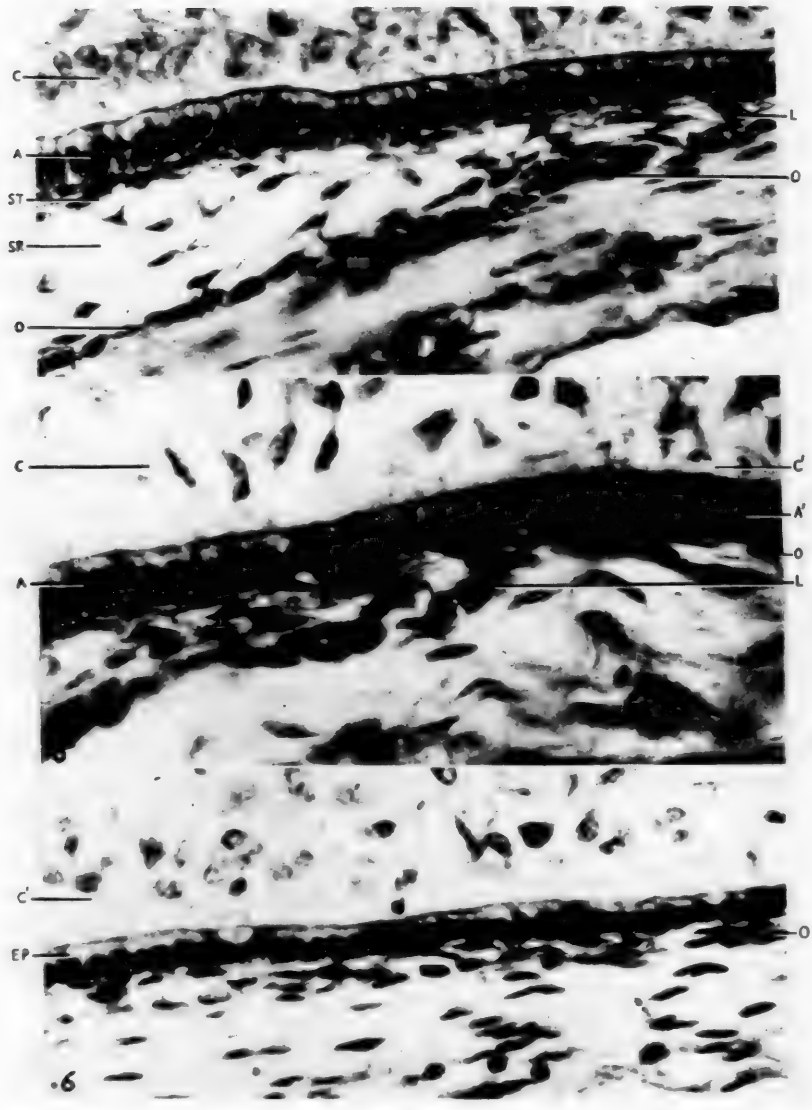

FIG. 4. Higher magnification of area enclosed within square #1 of *fig. 3*. C—undifferentiated connective tissue, A—undifferentiated ameloblasts, ST—stratum intermedium, SR—stellate reticulum, O—outer enamel epithelium, L—cervical loop.

FIG. 5. Area enclosed within square #2 of *fig. 3*. C—undifferentiated connective tissue adjacent to ameloblasts, C'—undifferentiated connective tissue adjacent to epithelial sheath, A—ameloblasts, L—cervical loop, A'—epithelial sheath, O—outer enamel epithelial cells.

FIG. 6. Higher magnification of area enclosed within square #3 in *fig. 3*. C'—undifferentiated connective tissue adjacent to epithelial sheath, Ep—epithelial sheath, O—outer epithelial cells of epithelial sheath.

connective tissue are undifferentiated. *Fig. 6* shows a continuous row of epithelial cells with the underlying adjacent connective tissue cells undifferentiated. On the other side of the row of epithelial cells, flattened oval cells are observed lying close to the epithelial layer.

DISCUSSION

The enamel organ is the formative organ for the crown of the tooth. At an early stage of tooth development, as presented in *fig. 1*, there is as yet no evidence of an epithelial sheath. The region generally designated as Hertwig's epithelial sheath (*fig. 1*), is instead the cervical terminal point of the enamel organ, and the eventual cemento-enamel junction of the tooth. With the beginning of hard tissue formation there occurs a considerable increase in the size of the tooth germ through a proliferation and stretching of the ameloblasts until they reach a state of functional differentiation as demonstrated in a previous study (2). The remaining cells of the enamel organ keep pace with the growth of the inner enamel epithelium.

The development of the crown of a tooth is practically completed before there is any evidence of root formation. With the advanced formation of enamel matrix in the incisal or occlusal regions, the enamel organ is steadily reduced. The enamel organ may be seen to remain intact in those regions where the enamel matrix has yet to begin formation or is still in an early state of formation. The presence of an enamel organ at any part of the developing tooth may be accepted as a criterion that the crown of the tooth is still in the process of development, and enamel is still to be formed. The direction of growth of the crown of the tooth is deeper in the jawbone; it grows in an eccentric pattern. The cervical loop of the enamel organ therefore shifts steadily deeper into the jawbone through a growth of the cells of the enamel organ, until the maximum size of the crown is reached. Apposition, necessarily, also increases the size of the crown in all directions. The cervical loop is therefore part of the enamel organ and cannot be regarded as a relatively fixed point (10, 11). The epithelial sheath becomes evident only in a much later stage of development, when most of the enamel has already been formed in a matrix state. This is seen in *fig. 2*, in which the maximum crown size has more or less been determined. The part of the enamel organ still seen intact in the cervical region of this developing tooth appears to be diverted inwardly in the direction of the dental papilla (*fig. 3*). This position of the enamel organ is undoubtedly an artifact, due to shrinkage and distortion, and it should lie in a more linear direction. The entire enamel organ narrows down in the direction of the cervical loop, at which point (*figs. 4* and *5*) the outer and inner enamel epithelium still appear in continuity, the inner enamel epithelium continuing beyond the cervical loop, and the cells of the outer enamel epithelium assuming a position close to the row of epithelial cells—a position formerly held by the stratum intermedium. The epithelial cells beyond the cervical loop, with the outer epithelial cells lying adjacent to them, may now be designated as the epithelial sheath.

At this stage (*fig. 5*) it is not possible to note any difference in morphology

between the epithelial cells on the enamel organ side of the cervical loop and those lying beyond the region of the cervical loop. It may be said with assurance that the epithelial cells on the enamel organ side will go on to their series of morphologic and physiologic changes to activate the formation of odontoblasts of the underlying connective tissue, and to secrete enamel. The epithelial cells beyond the cervical loop, however, will present a more limited series of morphologic changes, and will then activate the formation of dentin for the root of the tooth, without possessing the physiologic properties necessary to the secretion of the enamel.

The morphologic changes of the ameloblasts, described in a previous study by Diamond and Weinmann (2), prior to the state of functional differentiation, may be clearly seen taking place in the cervical region of the enamel organ. These morphologic changes (*fig. 3*) may be accepted as a criterion for their eventual functional differentiation in order to produce enamel. We maintain that these morphologic changes will take place with the epithelial cells as far as the cervical loop region of the enamel organ. The epithelial cells beyond the cervical loop present an entirely different pattern of behavior. While at this stage the presence of an epithelial sheath is already manifested, the cells of the epithelial sheath are still in an undifferentiated state, as evidenced largely by the absence of odontoblastic formation of the adjacent underlying connective tissue. The epithelial sheath will not become functionally differentiated until the formation of the enamel matrix is completed and the calcification process (1) is in progress. During this period, dentin continues to form within the crown as far as the cervical region. The formation of dentin within the crown is initially activated by the ameloblasts, whereas the dentin of the root is initially activated by the epithelial sheath. This will be demonstrated in a subsequent study.

CONCLUSION

It has been demonstrated that the epithelial sheath is a continuation of the outer and inner epithelium of the enamel organ, but that it does not become manifested until the formation of the enamel matrix of the crown is appreciably advanced, indeed not too far from completion. At this stage of development, however, the epithelial sheath is only outlining the contour of the future root, and remains in an undifferentiated state without activating the formation of root dentin until a later stage, when all of the enamel will have been completed in a matrix state of formation. It is therefore incorrect to designate the cervical loop of the enamel organ of a developing tooth germ at all stages of development as Hertwig's epithelial sheath. The cervical loop keeps shifting its position deeper in the jawbone until the maximum crown size is determined. The cervical loop may therefore be regarded as the future cervical region of the crown or the cemento-enamel junction. The cervical loop is not a relatively fixed point until the cemento-enamel junction has been attained.

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DENTAL STUDIES OF TRIPLETS[1]

II. Arch Form and Pattern

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It has been shown in the first part of this report that dental measurements taken at a specific time may lead to erroneous interpretations in the determination of monovular origin of the triplets. The purpose of this report is to show the degree of similarity found in the arch form and pattern of identical and non-identical individuals in sets of triplets. The conclusions drawn by Howard and by Rigler are accepted in the classifications as to the monovular origin (identical twins) of the members of the triplet sets.

METHODS

The arch patterns of the 9 sets of triplets and the 3 sets of non-related individuals whose dental measurements have been reported (Cohen, Oliver, and Bernick, 1942) are represented graphically. Cellophane which had been stretched over a rack was placed over the inciso-occlusal surfaces of the dental study casts and tracings were made of the surfaces of the teeth. The margins of the incisal and occlusal surfaces were represented by lines, and the cusps marked as points. Permanent premolars were represented by straight lines which connected the buccal and lingual cusps. Two lines were drawn for the molars; one representing a line from the mesial to the distal of the buccal cusps; the second connecting the 2 lingual cusps. If exfoliation or loss of the deciduous teeth had occurred, the approximate position of the cusp was marked. All the points were then connected, making an arch tracing of the incisal and occlusal margins of the upper and lower arches. The arches of a set of triplets were then superimposed for comparative study.

RESULTS

In the composite arch tracings the members of a set are designated by X, Y, and Z, the symbols representing corresponding individuals in these and in the dental measurement studies. For convenience, each triplet set is represented by a Roman numeral and each non-related set by an Arabic numeral corresponding to its triplet set.

I. Triplet set I was composed of 3 girls, 4 years old. In the tracings of the upper and lower arches, member X shows the widest and longest arches. The other members, Y and Z, have arches which are similar in general shape; but variations occur particularly in the upper arch with Z having in general the shorter and narrower arch of the two. In the lower

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[2] Due to an error which was not detected until after the article appeared, the degrees D.D.S., M.S. were included after the name of S. Bernick in Part I of this article; both degrees should have been omitted.

arch tracings, Y and Z show almost identical arch patterns. However, slight variations are observed in the right first deciduous molar segment and in the left side posterior to the canine.

II. The second set included 3 girls, 6 years of age. In the upper and lower arch tracings, member Z shows the widest and longest arches. Members X and Y, on the other hand, have similar posterior segments in the upper arches; but their anterior segments vary considerably, with the segment of Y being the wider and longer. In the left and right posterior regions, the lower arch of Y is similar in width and length to the corresponding areas of the arch of X; but the arch of Y has an anterior segment which is like the anterior portion of the

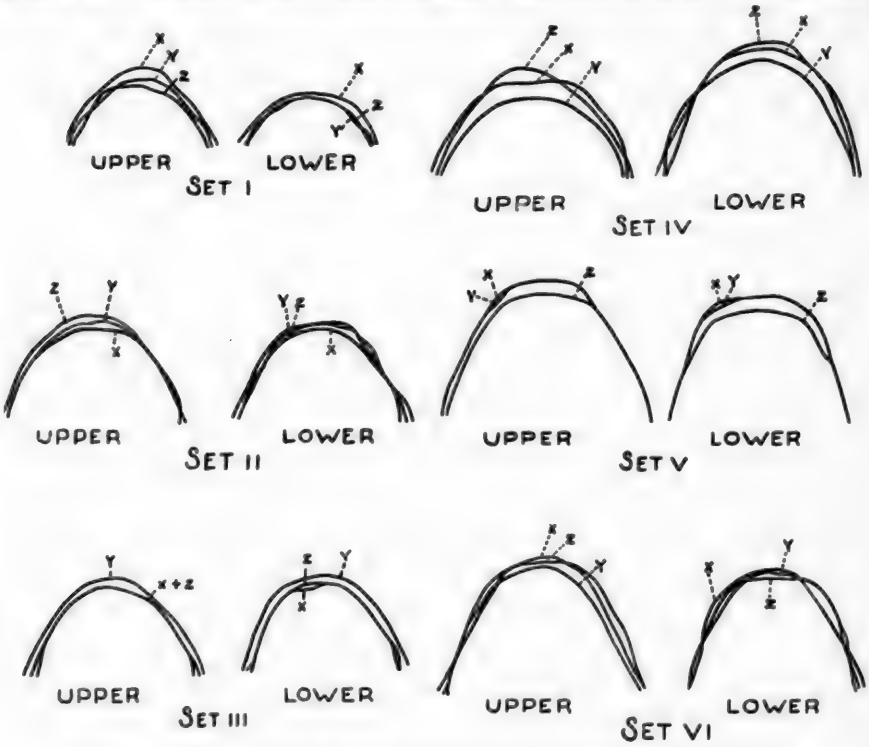

FIG. 1. Arch tracings of triplet sets and non-related sets of individuals

arch of Z in width and length. Members Y and Z had marked overbites in their upper jaws; but their arches vary in pattern, particularly in the anterior area.

III. The third set studied was made up of males, 6 years of age. Member Y shows the longest and widest upper and lower arches. In the tracings of the upper and lower arches, the forms of X and Z are almost identical. In the upper arch tracings, X and Z show a slight variation in the molar regions; and in the lower arch forms, variations occur only in the left second incisal area and in the right second deciduous molar region. The difference in the incisal area may be due in part to a labial rotation of the left second incisor. The great similarity in arch patterns between the monovular individuals X and Z is in agreement with the results of the dental measurements.

IV. The fourth set examined included 2 boys and a girl, 7 years of age. The upper and lower arch tracings of the 2 boys, Y and Z, vary considerably; the arches of the girl, X, are

more similar to the arches of one boy Z, than the arches of 2 boys are similar to each other. Member Y has the shortest and narrowest upper arch. In the lower arch tracings, Y has the shortest and narrowest arch except in the left molar region where the second deciduous molar had erupted buccally to the arch. The girl member, X, has the widest upper arch; but in length her arch is intermediate between Y and Z. The boy member Z has the longest upper and lower arch; however his arches are narrower than those of the girl, but wider than the arches of the other boy, Y. Although there had been mutilations of the arches, and differences in eruption time of the incisor teeth, the differences in arch patterns of the boys cannot be fully explained on that basis. The differences in pattern agree with the unlikeness found in the study of dental measurements and the conclusions of Howard and Rigler.

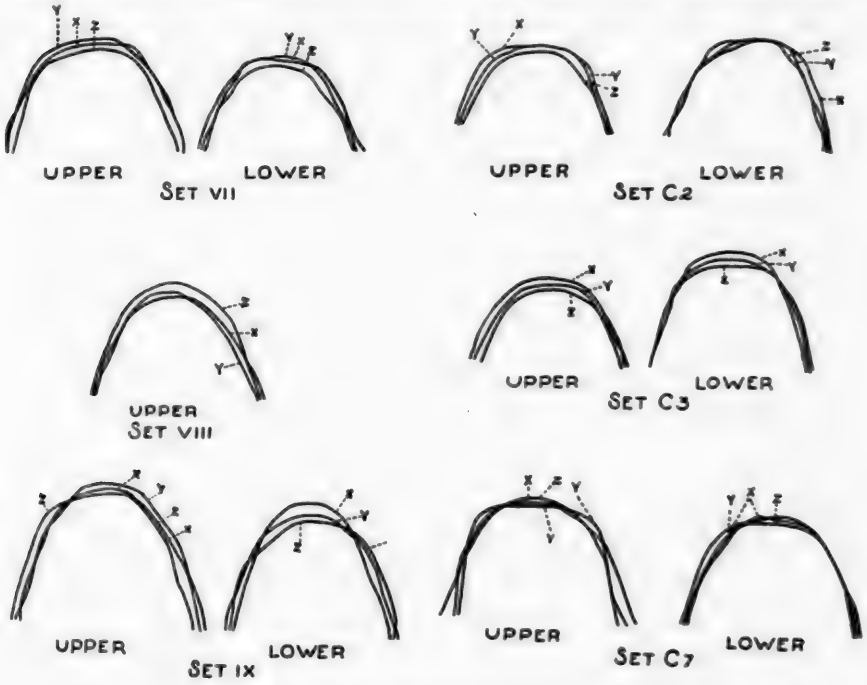

FIG. 2. Triplet sets are represented by Roman numerals; non-related sets, by Arabic. The symbols X, Y, and Z, represent the number of a set

V. The fifth triplet set consisted of 3 females, 9 years old. All 3 girls show arch tracings that are practically identical in the width and length of the molar regions. Anterior to the molars, member Z has the narrowest and shortest upper and lower arches. The upper and lower arch patterns of members X and Y are identical throughout except for slight variations in the upper and lower left second incisal regions. These variations are probably the result of a difference in the eruption time of the second incisors and of a labial rotation of the lower incisors.

VI. The sixth set consists of 3 girls, 9 years old, who were considered to be identical triplets by Lund (1933). Mutilations of the arches in all 3 sisters have caused considerable dissimilarity. Although there have been mutilations of the arches, the faint similarity found in arch form tracings for the 3 members is striking. In the upper arch tracings, member Y has the narrowest and shortest arch. The upper arches of X and Z are more or

less alike in general shape and size, although slight variations are observed throughout except in the right premolar region. The lower arch of member Y is the narrowest, but it is as long as the arch of X. The lower arches of X and Z have the same shape; but in size, the arch of Z is smaller in the incisal-canine length. On the left side, the lower arches of X and Z are alike for size and shape posterior to the canine. In the right posterior area, they show the same shape, but they vary in size in the premolar region. The narrowness of the arches of Y and Z in the upper tracings may be due to an early loss of the deciduous molars, which resulted in a tipping of the permanent molars in a mesial and bucco-lingual direction. The differences in the lower arches of X and Z are probably associated with a labial rotation of the incisors which had occurred in the arch of X; as a consequence, the arch tracings of member X is longer than the actual arch. The arch tracings accentuate the results found in the study of the dental measurements.

VII. The seventh set of triplets consisted of 2 boys and a girl, 13 years old. The girl, Z shows the narrowest and shortest upper and lower arch tracings. The boys, X and Y, have upper and lower arches that are nearly alike in general shape and size, but they vary slightly in pattern. Member Y has the longer and wider upper arch of the two. In the lower arch tracings of the 2 boys, variations are found in the incisal area where Y has a larger segment, and in the left premolar region with Y having the narrower segment. Certain factors to be discussed later may possibly explain the differences observed in the arches of the 2 boys. The arch patterns of the 2 boys do not agree with the results obtained from their dental measurements. In the tracings, the arches of the 2 boys are similar in general shape and size. In the study of dental measurements, the 2 boys were found to be the least alike of any set studied.

VIII. Two boys and a girl, 14 years of age, formed the eighth set. Due to mutilations in the arches, tracings were not obtainable for the lower arches. In the upper arch tracings, the girl member, Z, shows the widest and longest arch. The arch of one of the brothers, Y, is longer than the arch of the other boy, X. In width the 2 boys show somewhat similar arches particularly on the left side. The arches of X and Y seem to be more alike than they actually are; and that is due to the retention of the deciduous canines in the arch of Y (to be discussed later). The arch patterns agree with the conclusions drawn from the other studies of this set.

IX. Three girls, 16 years of age, were examined in set IX. Dissimilarities are observed in the tracings of the upper and lower arches. No 2 lines are alike, and no 2 arches have the same shape or size. Member X has the narrowest upper arch; but in length her arch is intermediate between Y and Z. The upper arch of Y is the longest of the 3 arches; that of Z, the widest. In the lower tracings, the arch of X is the longest and narrowest. Member Z has the widest and also the shortest lower arch. The arch of Y is intermediate in width and length. The individuals of this set had all their permanent teeth at the time of the study, and had no mutilations. Members Y and Z are less alike in arch patterns than they were in dental measurements.

C2. The first group of non-related individuals to be examined were 3 females, 6 years of age. They should be compared to triplet set II. In the tracings, member Z has the narrowest and shortest upper arch. Members X and Y have upper arches that are alike in length, but they vary in width. The variations between X and Y are found in the right side, with the arch of X being wider. In the lower tracings, member Z shows an arch which is as long as either of the other two; but it is the narrowest of the three. The arch of member X is wide on the right side and in the left canine region. As a whole the arch tracings of members X and Y are at least as similar in general size and shape as are the arches of members X and Y in set II who were classified as identical individuals by Howard and by Rigler.

C3. Three boys, 6 years of age, were selected for a comparative study with triplet set III. In the upper arch tracings, all 3 members show arches that are similar in general shape although they vary considerably in size. The arch of Z is the narrowest and the shortest; the arch of Y is intermediate in length and width; and the arch of X is the longest and widest. Member X has the narrowest and longest lower arch. The lower arches of

Y and Z are similar in width, especially in the posterior region. Variations occur in the anterior area, causing that segment in Y to be longer and wider. Although members Y and Z have arches which are similar in shape and, to a lesser degree, size, their arches are not as similar as those of the monovular pair in set III.

C7. The third non-related group, 2 boys and a girl, 13 years old, were used for comparison with triplet set VII. In the tracings, the upper arch of the girl, Z, is the widest in the posterior region, but it tapers to become the narrowest in the incisal area. It is, however, the longest of the 3 arches. The boy, Y, has almost the same upper arch form as the other boy X; but considerable variation can be observed in the canine region. The anterior segment of the upper arch of Y is wider and shorter than the corresponding segment in the arch of X. The girl, Z, has the narrowest and shortest lower arch. The 2 boys have lower arches which are similar in shape although slight variations occur throughout, and especially in the left premolar region. The differences in the anterior area of the lower arch may be due to the difference in eruption time of the premolars; at the time of the study, Y had his premolars, and X still had his deciduous molar. The arch patterns agree with the conclusions drawn from the dental measurements.

DISCUSSION AND CONCLUSIONS

Arch tracings representing the arch patterns of 9 sets of triplets and 3 sets of non-related individuals are shown. By selecting the pair of each set showing similarity in arch patterns, one can arrange the 12 sets from the set having arches most identical to the one showing arch forms most unlike; they are: V, III, I, II, C2, VI, VII, C3, C7, VIII, IX, and IV.

Of the 9 triplet sets studied, only sets V and III were found each to have 1 pair of numbers whose arch forms were nearly 1 line. In each set, variations were observed in only 1 region, and that variation was only temporary in nature. The similar arch forms were found in the numbers who were classified as "identical twins" by other criteria.

Each of sets II, VI, and VII was considered by Howard and Rigler to have 1 pair of monoval twins. In dental measurements, neither of the sets showed a degree of concordance as high as might be expected in identical persons. In the arch patterns, the identical members X and Y of set II showed variation. Their upper arches and to a lesser extent their lower arches were alike for width, but member Y had arches that were intermediate in length between those of her two sisters. A marked overbite in the upper jaw of Y was the probable cause of the observed difference in the length of the arches of the identical members of this set. The members of set VI were classified by Howard and by Lund as identical triplets. Only 2 members of this set showed any sort of similarity of the arches. The 2 members, X, and Z, had upper and lower arches that were more or less similar in general size and shape, but the pattern was dissimilar throughout. The variations that were observed might easily explained as due to the severe effect of mutilation on the arches of all 3 members. The 2 boys of set VII showed upper and lower arches that were similar in general shape and size although they differed in pattern. Slight variations which appear throughout the arch tracings were caused by extrinsic factors that are to be discussed. The degree of similarity of the arch tracings that one observes in this set is in marked contrast to the dissimilarity observed in the study of the dental measurements.

In the 4 triplet sets which were considered fraternal by other criteria, (I, IV, VIII, IX) the arch tracings of the members of each set vary in the degree of similarity. In set I, the 2 members Y and Z had lower arches that were nearly alike; their upper arches varied in size although the shapes were similar. At the young age of the set, 4 years, and with the lack of action of extrinsic factors, the observed similarity in shape of the lower arches was probably more apparent than real. Members of set IX showed considerable variation in arch form, just as they were found to vary in dental measurements. In set IV, the girl, X, had arches that were more similar to the arches of one of the boys than the arches of the 2 boys were like each other. The arches of the boy members varied considerably in width and length. Although mutilations may explain some of the differences observed in the arches of the boys, most of the differences in form and pattern are more basic in nature. In set VIII, the girl Z had the widest and longest arches of the three. The 2 boys had arches that were similar in width, although they varied in length.

In the group of non-related persons, members of set C2 showed no greater variation in arch pattern than did the members of triplet set II. The pair of individuals, X and Y, who had arches most alike in C2 showed only a dissimilarity no greater than that observed in the arches of the identical pair, X and Y, in set 2. Set C3 had 2 individuals, Y and Z, whose upper arches were similar in general shape, but not in size. Their lower arches were more or less similar except in the anterior region. The 2 boys, however, were not as similar in arch pattern as were the identical members of triplet set III. In set C7, the boy members, X and Y, had arch forms that were similar in general shape; but on comparison with the 2 boys in set VII the boys in C7 were more dissimilar.

ACTION OF EXTRINSIC FACTORS

Certain extrinsic factors may so alter the arches that one finds it difficult to interpret the arch tracings of twins. The effect of mutilation of the arches is best illustrated in set VI. Members of that identical triplet set should on the basis of heredity have arches of the same width and length. Due to the extreme carious condition of the permanent molars, and due to the early loss of the deciduous molars, the arches of the members were so deformed at the time of the study that only a faint similarity can be observed in the tracings of the upper and lower arches of 2 of the members, and the arch tracings of the third member, Y, are unlike those of her sisters. In set VII, the tracings of the lower arches of the boys X and Y show the effect of premature loss of the lower left second deciduous molar upon the dental arches. A collapse of the arches of both boys had occurred to such an extent that the succeeding premolars erupted lingually. As a consequence the adjacent teeth tipped in a mesial and distal direction and caused a change in the original length and width of the arches. At the time of the study the arch of Y, therefore, was narrower and longer than the arch of X. In set VIII caries of the first permanent molars in one of the boys, and also the early loss of the deciduous molars in the other boy caused changes in the arches which were so great that it was impossible to obtain tracings of the lower arches.

Another factor which can lead to misinterpretation in a study on identical twins is associated with the differences in the eruption time of the permanent teeth. Although that factor effects only a temporary condition in the arches, it does influence the width and length of the arches for a time. Differences observed in the arches of twins, if the comparison is made during the time of active change, may therefore be only temporary in nature. Four of our cases (sets IV, V, VII, VIII) illustrate this point clearly. In the identical twins of set V, members X and Y, the only variation in arch form was observed in the upper left second incisor region. The difference was probably due to the fact that the permanent left second incisor was still unerupted in one of the identical twins, and the corresponding tooth was in place in the arch of the other twin. As a result, one of the twins had at the time of this study a narrower anterior segment of the arch which should enlarge with the eruption of the permanent tooth. In set VII the deviation in the upper arches was found in the left molar region. One of the boys, Y, at the time the impressions were taken still had his left second deciduous molar; and X and Z had erupted all their permanent premolars. Lewis (1929), Smythe and Young (1932), and Cohen (1940) have shown that with the eruption of the permanent premolars an actual decrease in the length of the arches occurs. On that basis, the differences in the length and width of the upper arches of X and Y are explainable; the arch of Y was still in a temporary condition. In set VIII, the upper arches of the 2 boys were similar in width at the time the casts were made. This similarity was due to the retention of the deciduous canines in the arch of Y which caused his arch to be narrower than it would be if he had his permanent canine. The other sets showed similar pictures of differences in length and width which were due to the differences in eruption time of the permanent and deciduous teeth.

CONCLUSIONS

The arch patterns of identical twins, as shown in this study, were in general more alike than were the arches of non-identical twins or of non-related persons. Variations in arch patterns, however, can be expected in identical twins. Although each twin pair in triplet sets V and III were found to have almost identical arch patterns, considerable variations were observed in the identical twin pairs of triplet sets II, VI, and VII. Some of the differences observed in the arches of identical twins can be explained by mutilations and by differences in the time of eruption of the teeth. In set II, however, no mutilations had occurred at the time of the study; yet the pair of identical twins showed many dissimilarities.

Although identical twins are more likely to have similar arch patterns, the use of arch patterns as a means to determine mon-oval origin will likely lead to misinterpretations in some instances. In our study, the non-identical members of triplet set I had one pair of members who had arch patterns more nearly alike than some of the identical pairs in other sets. Also the non-related set C2 had one pair of members who were as similar in arch patterns as were the identical members in set II.

In the 3 sets of non-related members, 2 members of each set were at least as similar in arch patterns as were the members of the non-identical triplet sets. Individuals of like age and sex will often have relatively similar arch patterns regardless of heredity. Both methods used in this study, dental measurements and arch form and pattern, may give misleading information on mon-oval origin of individuals. Of the 2 methods, however, the use of arch pattern seems to give a more exact interpretation. In set IX, the 2 members with a relatively high degree of concordance in dental measurements had a high degree of variation in arch patterns. The 2 identical twins of set VII had the lowest degree of concordance of dental measurements of any of the sets studied; yet their arch forms were found to be more similar than those of many of the sets studied. Their arches were similar in shape and size, but varied slightly in pattern. The discrepancies in the conclusions drawn from the 2 methods of study are in part due to the differences in methods of study. The conclusion drawn from dental measurements were based upon exact values; those from arch patterns, upon general interpretations of likeness as based upon general shape and size of the arches.

SUMMARY

1. Tracings to show the patterns of the dental arches were drawn for the members of 9 sets of triplets and 3 sets of non-related individuals. 2. Five of the triplet sets had each 1 pair of identical twins, only 2 of the 5 pairs of identical twins showed identical arch patterns. The arches of the identical pairs in the other 3 sets were relatively similar in general shape and size, but they varied in pattern; however, for the most part they had arch patterns that were more alike than the patterns of the fraternal twins and non-related individuals. 3. The extrinsic factors which have caused the development of temporary or permanent differences between the arches of twins are discussed. 4. In the sets studied, the identical twins differed more in their dental measurements than they did in arch patterns. It is concluded that of the 2 methods of study, the use of arch patterns will be the more reliable in the determination of monovular origin.

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INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

SCIENTIFIC PROCEEDINGS OF THE NEW YORK SECTION[1]

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Compiled by FRANCES KRASNOW, PH.D., Editor, New York Section

Guggenheim Dental Clinic, New York, N. Y.

1. FAILURE TO PRODUCE DENTAL CARIES BY DISTURBANCE OF ACID-BASE BALANCE. *Bernerd O. A. Thomas*, M.S., D.D.S. and *Charles F. Bodecker*, D.D.S., Division of Oral Histology, School of Dental and Oral Surgery, Columbia University, New York, N. Y. (See J. D. Res., **21:** 1942.)

2. ELEVATED SALIVARY PROTEIN IN CARIES AND EROSION. *Frances Krasnow*, Ph.D., Guggenheim Dental Clinic, New York, N. Y. Salivary protein has been variously considered. Early data seemed to indicate somewhat higher values for caries-free individuals. Scrutiny with regard to "normal" cases has apparently reversed the finding. The series forming the basis of this report is the third indicating a similar trend. Analyses were made on both uncentrifuged and centrifuged unstimulated saliva from 260 subjects. The results show significantly higher values for the abnormal conditions with accentuation of divergencies for cases in which the dental disorder superimposed the systemic. Work in progress to ascertain the existence of differences in the stability of the protein for the several involvements points to a possibility of greater sedimentation in specimens of subjects suffering from caries or general body ailment.

3. ORIGIN OF ADAMANTOBLASTOMAS. *Edward V. Zegarelli*, A.B., D.D.S., School of Dental and Oral Surgery, Columbia University, New York, N. Y. Adamantinoma is defined as a "growth of adamantine or enamel-forming cells". However, since these particular tumors are characterized by a wide variety of epithelial cell forms which do not arise from enamel-forming cells, the term "adamantinoma" is a misnomer. A more acceptable term is adamantoblastoma. Our study is based on a group of 72 mice obtained from the Maude Slye colonies at the University of Chicago. All growths were hereditary. At the present time, popular theories explaining the site of origin of adamantoblastomas are: 1, residual epithelial cell nests; 2, proliferative disturbance of the enamel organ; 3, mucous membrane epithelium of the jaw; and 4, the epithelium of odontogenic cysts. In our mice, 97 pure adamantoblastomas were present, of which 87 showed unquestionable enamel organ origin, with no other source seen. In 8, downgrowths of epithelium at the site of oral ulcerations could have been designated as points of origin, but these tumors had enamel organ proliferations as

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well. Our study shows that the exact site of origin of the adamantoblastoma is the *outer or undifferentiated epithelial cells of the enamel organ.* We found that commonly designated "types" of adamantoblastomas, are not separate entities but stages in development. All stages are related and one may develop into another. We also found that adamantoblastomas are locally malignant with a slight potentiality to metastasize. We found evidence showing that the "follicular and dentigerous" cysts are developmental stages of adamantoblastomas.

4. AMELIORATION OF ESTROGENIC HORMONE EFFECT UPON THE ORAL MUCOSA OF RHESUS MONKEYS WITH LARGE DOSES OF VITAMIN A. *Daniel E. Ziskin*, D.D.S., and *Louis Drucker*, B.S., School of Dental and Oral Surgery, Columbia University, New York, N. Y. Long-term estrogenic therapy produces hyperplasia and hyperkeratinization. These changes were first reported as occurring in the vaginal epithelium during the treatment of gonococcal vaginitis in children. Later we reported similar changes in the oral mucosa induced experimentally and following estrogenic therapy for gynecological disorders. Experimentally induced Vitamin A deficiency was seen to have a like effect. The similarity in effect of estrogens and Vitamin A on oral epithelial structures raises question of the interaction of the two, if any. Published experiments on this subject have dealt chiefly with effects of Vitamin A on the estrous cycle; and rationalization of findings considered the metabolism of Vitamin A. Regarding the latter, the role of the thyroid was a factor. These studies also discuss results of estrogen on general metabolism, on local tissue metabolism, and its interrelationship with thyroid secretions. The present study deals with two sub-adult rhesus monkeys—one normal and one ovariectomized. After 1000 R.U. Progynon B for 53 days, biopsies showed epithelial hyperplasia and hyperkeratinization of gingivae. Subsequently, 10,000 U. of Vitamin A were added to Progynon B and the combination injected daily for 31 days. Sections of oral mucosa and vagina were studied. Findings: Estrogen alone produced marked hyperkeratinization and hyperplasia, as previously reported. Addition of Vitamin A disclosed suppression or amelioration of the estrogenic effect. Most obvious changes were in the areolar gingivae; but alveolar gingivae, cheek, and vaginal mucosal epithelium were clearly affected.

5. A STUDY OF THE EFFECT OF EXTRA-ORAL TRAUMA ON THE TOOTH AND SURROUNDING STRUCTURES. *Oscar Miller**, D.D.S., *Morris Cohen**, D.D.S., and *Charles G. Darlington*, M.D., College of Dentistry, New York University, New York, N. Y. Forty-six selected patients who have had some previous extra-oral trauma were studied and reviewed from a clinical and histopathological standpoint. Some of the conclusions reached were: 1, The ability of the tooth and surrounding structures to repair injuries varies in the individual; 2, Infection and inflammation and the ability of the pulp to repair cannot be pre-determined by the amount of trauma; 3, Healing in tooth fractures beneath the gum line is primarily accomplished by the components of the periodontium; 4, Some of the results—in the periodontium but not in the pulp—can be compared with repair following trauma to the long bones.

*Guest.